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# A REVISION OF THE MOVEMENTS OF THE FOREARM IN TETRAPODS

By R. WHEELER HAINES, *St Thomas's Hospital Medical School*

## INTRODUCTION

The evolution of the bones of the forearm has been studied in detail by a number of authors in both living and fossil material, and the muscles, well known in living types, have been reconstructed for some earlier types. The elbow joint has been carefully studied in a large and representative series of animals by Hultkrantz (1897), whose work includes full references to the earlier literature, and can be used as a basis here. Parsons, in his independent work on the joints of mammals (1900), and in his studies of particular types, gives good accounts of the elbow and radio-ulnar joints of several forms. Fick (1911), with Hultkrantz's help, published a detailed table summarizing the structure and movements of the elbow joint in the various groups of tetrapods, and the work is again summarized by Nauck (1938) with little change. It may be said that the elbow joint has been more fully described and discussed than any other.

Even so the evolution of the bones and joints has been misunderstood. At the time Hultkrantz wrote, few of the early tetrapods were available for study, and, though he made use of fossils in his work on ungulates, his evolutionary scheme is based essentially on living urodeles and monotremes, both unsuitable forms for this treatment. Hultkrantz was chiefly, and Parsons exclusively, interested in mammalian mechanisms, so that the reptilian structure is but poorly known. In this revision, living reptiles and amphibians will be considered in some detail and the conclusions will be checked by a reference to fossil forms. Mammals will receive a more cursory treatment, and emphasis will be laid on more primitive types.

*Movements of the bones.* By pronation and supination are understood movements of the hand or forefoot about an axis passing along the forearm, which are brought about by displacements of the radius and ulna in relation to each other. It will be seen that all primitive tetrapods had such movements, and that the majority of living forms have them still, though details of the mechanisms by which they are brought about differ in the various groups. The human type of mechanism represents an extreme specialization, but the terms pronation and supination can be applied more widely.

The movements involved in pronation and supination can be analysed into three main categories: (1) the displacements of the radius and ulna relative to each other; (2) the rotations of the hand about the axes of each of these bones; (3) the rotations of the radius and ulna about their own long axes at the elbow joint. The displacements (1) are similar in all animals in which pronation and supination occur, but as the hand moves, the radius may rotate with it, so that this rotation is transmitted to the elbow joint, a mechanism perfected in man, or, alternatively the hand may rotate about the radius, a mechanism well developed in lizards. Likewise, rotation may occur wholly between the hand and the ulna as in man, or may be transmitted in part to the elbow. Thus the movements of rotations (2) and (3) are complementary, and it will be seen that in most animals movement of each bone occurs both at the wrist and the elbow.

The mechanical problems involved in pronation and supination are similar to those of rotation of the lower leg in animals in which both tibia and fibula articulate with the femur (Haines, 1942*a*). In the hind limb the difficulty of providing a stable ankle joint has been solved in eutherian mammals by the complete loss of the articulation of the fibula with the femur and its close attachment to the tibia, so that the tibia and fibula move together. In the forelimb, however, both the radius and the ulna have retained their articulations with the humerus, so that the movements in the two limbs are no longer closely comparable.

## REPTILES AND AMPHIBIANS

*Sphenodon punctatum.* This form probably possesses the most primitive type of elbow-joint found in living animals. The lower end of the humerus carries an obliquely placed radial condyle (*cnd.rd.*) separated by a deeply cut intercondylar groove (*gr.in.cnd.*) from an ulnar condyle (*cnd.ul.*). This ulnar condyle is separated by a shallower groove (*gr.ul.*) from an ulnar facet (*fc.ul.*) developed on the overhanging medial epicondyle. The head of the radius is oval with a deep hollow for articulation with the radial condyle, and has a slightly convex facet for the radial notch of the ulna. The ulna has two ridges on its sigmoid surface, the one articulating with the intercondylar groove (*rg.in.cnd.*), the other with the ulnar groove (*rg.ul.*); between these there is a hollow for the ulnar condyle of the humerus. Thus, while the radius articulates with the radial condyle only, the ulna articulates with the ulnar condyle and, by two special facets, with the medial surface of the radial condyle, and with the facet on the medial epicondyle of the humerus.

The collateral ligaments of the elbow joint (*l.col.rd.* and *ul.*) are weak, and can play little part in the maintenance of the stability of the joint, which depends chiefly on the pull of the flexor and extensor musculature of the upper arm. The radio-ulnar ligaments (*l.rd.ul.s.f.* and *e.*) are strong, and, though they are lax enough to allow a small amount of independent movement of the radius and ulna, such movements are very limited in extent. Between the elbow and wrist the radius and ulna are quite free, for there is no interosseous membrane. The flexor and extensor muscles are separated only by a thin layer of loose connective tissue, similar to that separating the individual muscles of each group, and by the large nerve trunk which passes from the flexor to the extensor surface of the limb to supply most of the extensor muscles. At the wrist the radio-carpal and ulno-carpal joints are quite separate, and their ligaments are independent. The radius is firmly attached to the radiale by a strong interosseous ligament, which allows the radius to rotate a little on the carpus but limits other movements. The ulna articulates by a wide convexity with the ulnare and pisiform, and is attached to the bones of the proximal row of the carpus by ligaments (*l.ul.cp.*). The pisiform is attached to the ulna by piso-ulnar ligaments (*l.ps.ul.*), and to the bones of the carpus by piso-carpal ligaments (*l.ps.cp.*). The carpal bones are closely bound together, particularly anteriorly, but the ligaments allow some movement between the individual bones, more especially flexion and extension at the mid-carpal joint. The intermetacarpal ligaments are well developed on both volar and dorsal surfaces, and are rather taut; so that, when either the fifth or the first metacarpal is abducted from the median plane of the hand, the neighbouring metacarpals are pulled on by the series of ligaments and deviate in the same direction. Radial and ulnar deviation of the hand at the wrist as found in man cannot occur in *Sphenodon*, since the radius and ulna are too firmly attached to the radiale and ulnare, yet the same functional result is obtained by deviation at the mid-carpal and carpo-metacarpal joints.

Pronation and supination amounting to about 45° are possible; these can be supplemented to a slight extent by carpal and carpo-metacarpal movements. As the hand turns, most of the rotation is transmitted along the radius to the elbow, although some is taken up at the radio-carpal joint. Though the ligaments of the superior radio-ulnar joint are strong they are lax and allow a fair range of movement. The carpus can, on the other hand, rotate freely about the expanded lower end of the ulna, but the ulna itself rotates to some extent, so that a little of the movement is transmitted to the elbow joint.

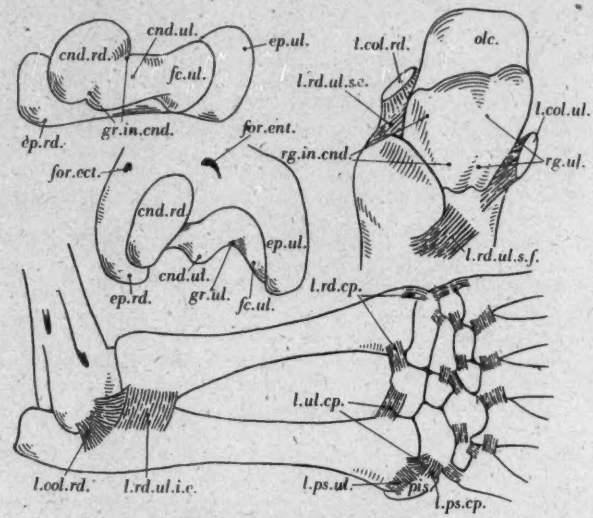


Fig. 1. *Sphenodon punctatum.*

Hultkrantz (1897) described briefly the radial and ulnar condyles and the overhanging ulnar facet on the humerus; he did not see the facet on the ulna for the radial condyle, and described this condyle as articulating only with the radius. Besides movements of flexion-extension and pronation-supination he and Fick (1911) described in *Sphenodon* (and other reptiles) lateral movements of the forearm at the elbow, combined with a longitudinal displacement of the radius and ulna relative to one another, so that the hand was deviated to one side

or the other as the forearm moved. In my specimens, however, these movements are very limited in extent, for the ulna is rather firmly locked into position at the elbow by its two ridges which engage with the two grooves on the humerus. Again, the effect of a lateral movement at the elbow would be functionally equivalent to a rotation at the shoulder; this rotation is possible in *Sphenodon* and lizards. It will be seen later, that, as Watson (1917) suggested, there may be good reason to postulate a lateral movement at the elbow in primitive fossil reptiles, but no direct evidence of any considerable movement can be found among living reptiles.

*Varanus niloticus.* The structure of the forelimb of lizards is similar to that of *Sphenodon*, so that only the differences need be discussed. The humerus carries well developed radial and ulnar condyles separated by an intercondylar groove, but no

Other lizards such as *Ameiva* (Hultkrantz, 1897), *Iguana* (Romer, 1922 and personal observations), *Conolophus* and *Agama* agree very closely with *Varanus*. The points wherein the lizards differ from *Sphenodon* all appear to be specializations associated with an increase in the degree of freedom of pronation and supination, and there is every reason to suppose that the ancestral group which gave rise to the Rhynchocephalia and Lacertilia was *Sphenodon*-like in the structure of the forearm.

*Terrapene carolina.* In this turtle the radial condyle is rounded, the ulnar condyle is more cylindrical, and the intercondylar groove well developed. The ridge which articulates with the groove is, as in *Sphenodon*, confined to the ulna, so that the radius has a simple concavity for the radial condyle, while the ulna has facets for both condyles.

The collateral ligaments of the elbow joint are

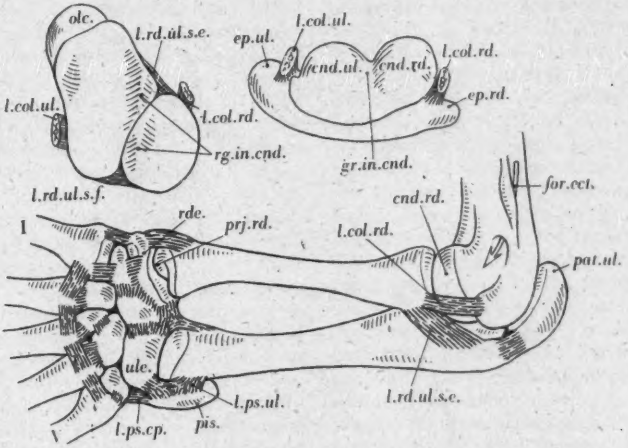


Fig. 2. *Varanus niloticus.*

special ulnar facet. The ridge that engages with the intercondylar groove (*rg.in.cnd.*) crosses both ulna and radius, so that each of these bones articulates with both condyles. There is a bony ulnar patella in the tendon of the triceps. At the radio-carpal joint there is no interosseous ligament in the interior of the cavity, but the radius carries a hemispherical projection (*prj.rd.*) which engages with the socket on the radiale.

Pronation-supination movements are free, amounting to about 60°. This agrees with Hultkrantz's (1897) account, but not with Fick's (1911) table. Since the radio-carpal joint is more freely movable than in *Sphenodon*, some of the rotation of the carpus is taken up at this joint. At the elbow the ulna is rather more movable than in *Sphenodon*, for its articular surfaces are not so complex, and some of the rotation of the carpus is transmitted to the elbow.

thin wide structures of little strength. On the other hand, though the shafts of the radius and ulna are, as in all primitive reptiles, bowed away from each other, the bones are firmly connected at both ends by radio-ulnar ligaments, not at the upper end only as in *Sphenodon*. There is no actual joint between the radius and ulna at the wrist, and the articular cavities of the radio- and ulno-carpal joints are quite separate. The intermedium is held firmly between the lower ends of the radius and ulna by radio- and ulno-carpal ligaments, so that in flexion and extension of the wrist it moves with the forearm bones. The proximal and distal rows of carpal bones have become aligned to give great mobility of the midcarpal joint, a peculiar specialization, possibly aquatic, found in other Chelonia.

Pronation and supination are limited to a total movement of about half a right angle, sufficient to adapt the hand to the ground in walking. As in

lizards, rotation both of the carpus about the lower ends of the radius and ulna, and of the upper ends of the radius and ulna about the humerus, occur, but to a more limited extent.

Other Chelonia examined (*Testudo*, *Chelydra*, *Sternothaerus*) agree in structure with *Terrapene*, but in *Chelydra* the ridge that engages with the intercondylar groove of the humerus crosses both the radius and the ulna as in *Varanus*, instead of figured by Hultkrantz, and my own specimens of this genus, *Crocodilus* and *Caiman*, agree well with his description, but the structure is very peculiar and need not be discussed here. The birds discussed by Fürbringer (1886) and Hultkrantz have radio-ulnar movements adapted for folding of the wings.

*Salamandra maculosa*. The radial condyle is large and spherical, the ulnar condyle relatively poorly developed; and the intercondylar groove is not so

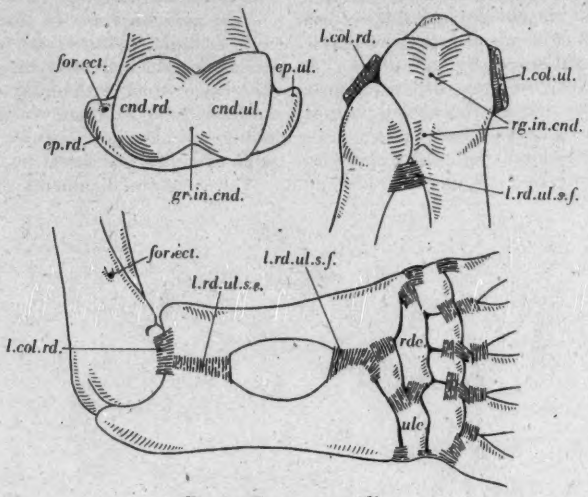


Fig. 3. *Terrapene carolina.*

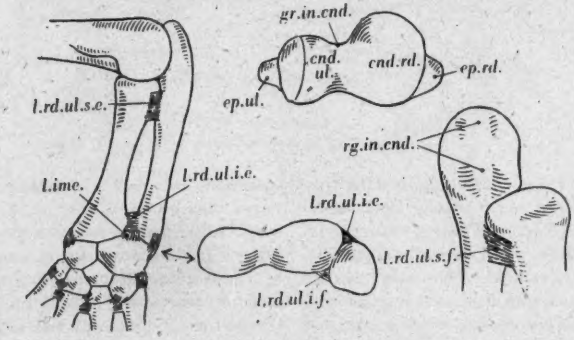


Fig. 4. *Salamandra maculosa.*

being confined to the ulna as in *Terrapene*. Hultkrantz (1897) found no pronation or supination in the chelonians, of which he examined *Testudo graeca* and *Chelone viridis*. Both these forms are atypical, for *Chelone* has limbs highly specialized as paddles, and *Testudo graeca* has shortened digits and a peculiar gait. My specimen of *Testudo loveridgii*, a rock-crevice dweller with less specialized limbs, showed undoubted movements of pronation and supination. Of the crocodilians, *Alligator* has been sharply cut as in the reptiles described. The ridge that engages with the groove (*rg.in.cnd.*) crosses the sigmoid surface of the ulna to divide it almost equally into parts articulating with the ulnar and the radial condyles. There are no distinct collateral ligaments at the elbow, but the superior radio-ulnar ligaments are well developed. At the wrist the radius and ulna articulate by a joint the cavity of which is continuous with that of the wrist joint; its histological structure has been studied by Schwartz

(1935). This joint has ligaments on its flexor and extensor surfaces (*l.rd.ul.i.f.* and *e.*). Besides the radial and ulnar collateral ligaments at the wrist joint, there are intermediate ligaments (*l.ime.*) passing from the radius and ulna to the compound ulnare-intermedium.

As in turtles, pronation and supination are limited to about half a right angle. Each of the six articulations in which the radius and ulna take part allows a little movement as the carpus turns, particularly the ulno-carpal and humero-radial joints. Other urodeles examined (*Necturus*, *Cryptobranchus*) agree well with *Salamandra*, and Miner's (1925) detailed drawings of the articulated bones of *Megalobatrachus* show a similar arrangement.

It will be seen that *Salamandra* is not considered here to differ in its essential structure or in its movements from the reptiles already described. But Hultkrantz (1897) and Fick (1911) described a very different series of mechanisms. They found in *Salamandra*, as in frogs, where the radius and ulna are fused, no pronation-supination movements whatever, but a wide range of lateral movement, coupled with some rotation of the radius and ulna together, and with a considerable longitudinal displacement of the radius and ulna relative to each other, leading to a radial or ulnar deviation of the wrist. But, as in *Sphenodon* and lizards, though the intercondylar groove and the ridge that articulates with it may not be so sharply cut, they would appear to be sufficient to prevent any considerable degree of lateral movement when the muscles controlling the forearm movements are active. Schaeffer (1941), on the other hand, from a study of cinematographical records of the gait, and of cleared specimens with alizarin stained bones, described pronation-supination movements of very great range in urodeles, well over a right angle, leading to a complete crossing of the radius and ulna at the end of each stride. Such movements would however be quite impossible without dislocation of the joints, nor would they appear necessary to account for the gait of the animal, which, as is shown so well in Schaeffer's figures, is of the ordinary tetrapod type.

*Early Amphibia and Reptilia.* It has been seen that *Sphenodon* and *Varanus*, *Terrapene* and *Salamandra* all have their joints constructed on the same general plan, though there is variation in detail, that the movements are similar though differing in amount, and that these animals are fairly typical of the groups of Amphibia and Reptilia to which they belong. It remains to show, as far as can be done from bony remains, that the early representatives of these groups had similar anatomical arrangements, and that there is little evidence that the movements were very different in these primitive forms.

Unfortunately the animals which would otherwise be the most suitable for study, such as the small amphibian *Diplovertebron* or the primitive cotylosaur *Seymouria*, had large cartilaginous epiphyses, so that the shapes of the articular surfaces are unknown (Haines, 1942*b*). Thus it is only from the later and more specialized types, in which ossification has spread to the neighbourhood of the articular surface, that evidence may be obtained. Since the structure is uniform over a wide range of animals, and is in good agreement with modern forms, it seems probable that the articular surfaces had the usual shapes, even where they remained cartilaginous.

The amphibian *Eryops* (Romer, 1922; Miner, 1925) and the reptile *Ophiacodon* (Williston & Case, 1913) are both represented by well-ossified and well-preserved individuals. In each animal there is a large hemispherical radial condyle projecting from the distal end of the flexor surface of the shaft, separated by an intercondylar groove, better developed in *Ophiacodon*, from a flatter, more cylindrical ulnar condyle situated on the end of the shaft, and extending equally on to both flexor and extensor surfaces. In the amphibians *Mastodonsaurus* (Huene, 1922) and *Achelomma* (Romer, 1922) the radial condyle is unossified, but the surface of endochondral bone that carried it is clearly defined, and the presence of an intercondylar groove in the living animal is indicated by an incision in the periosteal bone on the flexor surface. In the diadectid reptile *Diasparactus* (Case & Williston, 1913) the position of the radial condyle is clearly marked as a nearly circular facet in the usual position, but the actual surface is flat or concave, indicating, according to the authors quoted, the presence of a radial condyle of the familiar hemispherical shape, but unossified. On the other hand, Watson (1917) found in *Dicynodon* a similar flattened bony condyle and suggested that it indicated a limitation of the movements of flexion and extension at the elbow, but it seems more probable that as in *Diasparactus* the condyle was rounded, but not fully ossified.

The evidence from the fossils mentioned, and from the long series figured by Romer (1922), is uniform and in complete agreement with that from living forms. At the elbow the radius always articulated with the radial condyle, which lay on the flexor surface of the shaft, and was separated by an intercondylar groove from the ulnar condyle on the end of the shaft. The ends of the radius and ulna are not usually sufficiently well ossified or preserved in fossils to show the exact position of the ridge which engaged with the intercondylar groove, but in *Eryops* (Miner, 1925) there is a bony ridge on the ulna which probably represents this structure. The over-hanging medial epicondyle and special ulnar facet in *Sphenodon* is undoubtedly a peculiarity, though a somewhat similar arrange-

ment has been found in *Diadectes* (Romer, 1922). At the wrist the radius and ulna are, in all primitive forms, widely separated, so that *Salamandra* and other living urodeles are peculiar in the possession of an inferior radio-ulnar joint. There is never any trace of interosseous ridges that might indicate the presence of an interosseous ligament between the radius and ulna.

The movements are difficult to assess. Flexion-extension was certainly free, and, since the articular surfaces of the humerus and the arrangement of the radius and ulna resembled closely those of living reptiles such as *Terrapene* or *Sphenodon*, there is little doubt that pronation-supination amounted to about half a right angle; and this would seem necessary to adapt the hand to uneven ground.

Watson (1917) believed that in early tetrapods movement at the shoulder was limited to a single, nearly horizontal line, and that, to compensate for this limitation, the elbow allowed not only flexion and extension but also ab- and adduction in a plane transverse to the axis of the humerus. Romer (1922) has criticized Watson's interpretation of conditions at the shoulder, though neither he nor Miner (1925) has stated clearly what he thought the movements really were. At the elbow there is little evidence of ab- or adduction in the shapes of joint surfaces, nor would the footprints of *Ammobatrachus*, beautifully figured by Schaeffer (1941) imply the presence of such movements. In living animals they are known only among such specialized forms as the monotremes, edentates and moles (Hultkrantz, 1897).

## MAMMALS

*Dasyurus viverrinus*. The mammalian mechanism differs from the reptilian in many points. The lower end of the humerus, instead of carrying two distinct surfaces separated by a sharply cut groove, has a relatively wide trochlear groove (*troch.*) which is only partially separated by a slight ridge (*rg.cap.*) from the capitular surface for the radius (*cap.*). The ulna has a sigmoid surface which expands symmetrically on either side, and is widest near the olecranon. The head of the radius is round rather than compressed, and is set off from the shaft by a distinct neck.

The radius and ulna lie parallel and close to each other, instead of being bowed away from each other as in reptiles, and they are bound together by a strong interosseous ligament (*l.int.o.*) throughout the greater part of their length. Where the shafts of the bones deviate from the line of this ligament the bones develop flanges, the ulna proximally (*ul.fl.*) and the radius distally (*rd.fl.*) so that the interosseous margins are close together, and the fibres of the interosseous ligament attached to these flanges remain short. This ligament, even when all others are cut, enforces a rigid hinge movement between the radius and ulna about an axis which passes along the ligament itself and through the middle of the head of the radius.

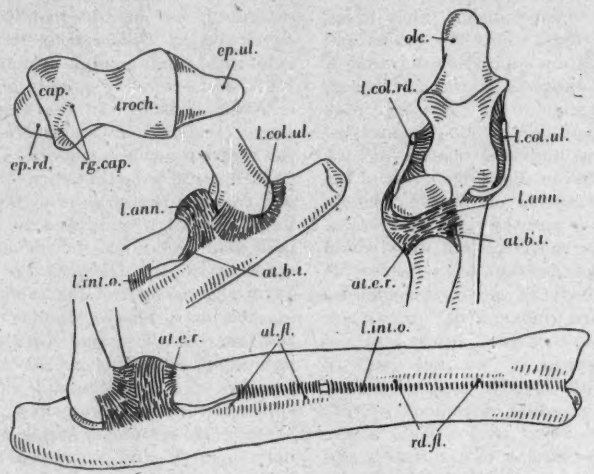


Fig. 5. *Dasyurus viverrinus.*

The radial and ulnar collateral ligaments are wide and each is attached partly to the ulna and partly to the annular ligament. The annular ligament (*l.ann.*) is attached to the ulna at the margins of the radial notch, and also to the radius, both on its extensor surface in line with the radial collateral ligament (*at.e.r.*), and on its flexor surface, to the bicipital tuberosity (*at.b.t.*). At their lower ends the radius and ulna are closely bound together, but there is no joint cavity between them.

Supination is freer than in most reptiles, amount-

ing to about one right angle. The carpus takes the radius with it, so that most of the rotation is at the elbow. The ulna has a distinct rounded styloid process about which the carpus rotates, but some of the rotation is transmitted to the elbow. The posterior part of the sigmoid cavity of the ulna is wider than the surface on the humerus with which it articulates so that, as the ulna rocks during pronation and supination, the expansion on one side or the other comes into full articulation with the humerus. Associated with these main movements of pronation and supination are some accessory movements, due to the indirect attachments of the collateral ligaments to the radius. These movements will be studied in detail in the cat, where they are more highly developed.

In other marsupials the articular surfaces resemble those of *Dasyurus* (Hultkrantz, 1897, *Perameles*, *Didelphys*, *Myrmecobius*). The annular ligament is absent in *Didelphys* (Parsons, 1900, *Didelphys virginiana*; personal observations, *D. philander* and *murina*), but is found in most genera (Parsons, 1900, *Macropus*, *Petrogale*, *Perameles*; 1903, *Choeropus*), though Hultkrantz did not notice this structure in any of the forms he examined, which included *Perameles* and *Macropus*. My own observations support Parsons's (1900) contention that whenever, in any mammalian phylum, the movements of pronation and supination approach a right angle an annular ligament is developed.

Hultkrantz suggested that the interosseous ligament, here considered as of some importance in the mechanism of supination, was a variable structure in marsupials. In *Didelphys* he described only isolated bundles, but my own specimens show a strong continuous ligament similar to that figured in *Dasyurus*. Thus it seems probable that the parallelism of the radius and ulna, their attachment by a strong interosseous ligament, and their movement about an axis which passes along the ligament and through the rounded head of the radius, are all primitive features of the marsupial structure.

*Sciurus carolinensis* and *Felis domestica*. The mechanisms in these two animals are, as Hultkrantz (1897) has pointed out, closely similar, and since the mechanisms found in many other animals appear to have been derived from this type, it seems reasonable to assume that this is primitive for eutherian mammals in general.

The surfaces of the bones resemble those of *Dasyurus*, but the ligaments are more highly differentiated. In *Felis*, using Hultkrantz's terminology, there is a strong epitrochleo-anconeal ligament from the medial epicondyle to the olecranon (*l.etr.an.*) and, as in most mammals, an epitrochleo-coronoidal (*l.etr.co.*). The epitrochleo-

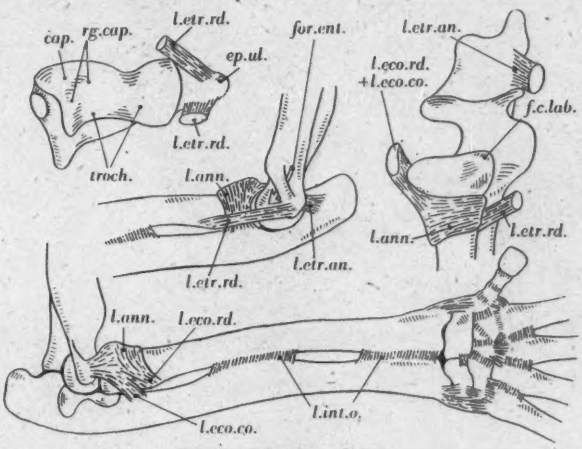


Fig. 6. *Sciurus carolinesis.*

radial ligaments are present in both *Felis* and *Sciurus* (*l.etr.rd.*) though in the case of the latter it is more separate from the other ligaments. From the lateral epicondyle pass an epicondylo-coronoidal (*l.eco.co.*) and an epicondylo-radial ligament (*l.eco.rd.*). An annular ligament (*l.ann.*), largely formed of fibres from the epitrochleo- and epicondylo-radial ligaments, surrounds the head and part of the neck of the radius. The radius and ulna lie close together and are bound by a strong interosseous ligament (*l.int.o.*), interrupted in part of its extent in *Sciurus*. In *Felis*, as in other carnivores (Parsons, 1900), there is a well developed inferior radio-ulnar joint, separated from the wrist joint by a disc-like formation of dense fibro-vesicular tissue.

After the removal of all connections except the ligaments the bones are still held in rigid apposition to one another. Flexion and extension are free,

while pronation and supination amount to about a right angle. The radius rotates with the hand.

In *Felis* the radial styloid process articulates with a groove in the scapho-lunar (*gr.sty.*) which prevents any antero-posterior displacement, and the interosseous and annular ligaments restrict its movement to a simple rotation. The ulna does not rotate with the hand to the same extent as does the radius, but the movement at the upper end is still quite appreciable, and appears as a rocking of the sigmoid surface over the trochlear surface of the humerus.

The rigidity of the elbow joint depends on the epitrochleo- and epicondylo-radial ligaments. They are inserted close together on each side of the bicipital tuberosity of the radius, so that as the radius rotates in supination the epicondylo-radial ligament is wound up and the epitrochleo-radial played out, and if the humerus is held fixed, the radius is abducted; similarly it is adducted when the arm is pronated. This mechanism was well described by Hultkrantz, who supposed it to be of use in tree-climbing. The ligaments are similarly arranged in other carnivores (Hultkrantz, 1897; Parsons, 1900) and rodents (Parsons, 1898, *Pedetes*; *Mus decumanus*, personal observation), and there is a similar mechanism, though less well developed, in the marsupial *Dasyurus*. In specialized cursorial mammals, in which the movements of pronation and supination have been lost, the elbow joint approaches more closely to a hinge mechanism and the ligaments are less well developed. In view of the full development of the complex set of ligaments in carnivores and rodents, and of similar formations in lemurs and monkeys, it is believed that the joints described in *Felis* and *Sciurus* are of the primitive eutherian type, and this would agree well with the conclusions of Matthew (1904) and Simpson (1937) on the movements in primitive mammals, based on palaeontological evidence. Hultkrantz was concerned to establish the typical rather than the primitive mammalian structure, but his work would agree with the suggestions put forward here, except that he did not consider the interosseous ligament to have any particular significance. Fick (1911) has given a very comprehensive survey of the effect of running, swimming, digging and other habits on the elbow, but these need not concern us here.

*Monotremes.* In both *Ornithorhynchus* and *Echidna* the radius and ulna are bound together by an interosseous ligament which prevents any considerable amount of pronation or supination. In *Echidna* (Hultkrantz, 1897 and personal observations) the articular surface of the humerus has a large rounded condyle about which the ulna and radius move, and a deep groove on the radial side, overhung by a malleolus-like formation, with which the radius articulates by a special bevelled facet. The superior radio-ulnar surfaces are flat to allow the radius to be displaced laterally relative to the ulna, but not to rotate on it. Besides flexion and extension, the forearm bones are capable of rotation as a unit at the elbow, the displacement of the radius allowing it to maintain contact with the special facet on the humerus. With rotation there is combined a lateral movement of the forearm, but any attempt at an independent lateral movement of ab- or adduction (suggested by Hultkrantz as normal), leads at once to a dislocation of the radius from its groove, or of the concave radio-ulnar facet from the main convexity of the humerus.

In *Ornithorhynchus* the forearm bones are more closely bound together. The elbow joint is of the ball and socket type (Howell, 1936), and plays an important part in the swimming mechanism, not

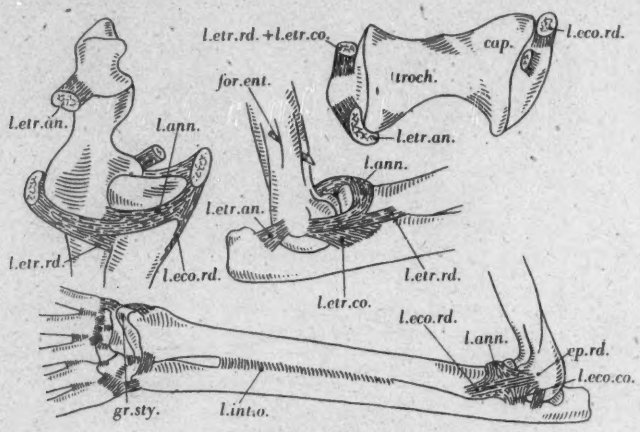


Fig. 7. *Felis domestica.*

as Parsons (1900) states, of the hinge type. It is clear that neither of the living monotremes can throw any light on the history of radio-ulnar movements in early placentals or marsupials, though they appear to possess a mechanism specialized from the typical mammalian condition, with a well developed interosseous ligament.

*Mammal-like reptiles and early mammals.* Most mammal-like reptiles show variations of the general reptilian type, but some of the more specialized forms suggest an evolution towards a mammalian type of mechanism. In *Dicynodon halli*, figured by Watson (1917) from a specimen in which the bones were in natural articulation, the radius and ulna were no longer bowed away from each other as in most reptiles, but were parallel as in typical mammals. In *Galesaurus* sp., described by Parrington (1934), the shaft of the ulna had a well developed crest, which is said to have faced anteriorly, but it seems more likely that it faced laterally and was associated with the attachment of an interosseous ligament.

The Stonesfield humerus (British Museum, No. M13127) from a Mesozoic mammal described by Simpson (1928) is, however, still reptilian in form. There are two eminences, a radial and an ulnar condyle, separated by a deep narrow groove, so that the whole articular surface can be compared directly with that of *Diademodon*, a mammal-like reptile, or with a modern lizard, such as *Varanus*. This humerus demonstrates the probability that the wide mammalian trochlear surface has been derived by the opening out of the groove between the pair of reptilian condyles, and that the mammalian capitulum was formed from but a part of the radial condyle, the remainder forming the lateral wall of the trochlear surface. The position of the secondary centres of ossification in the mammalian humerus also supports this thesis, for the centre for the capitulum helps to form the trochlear surface.

The Palaeocene mammals (Matthew, 1937) already possessed a modern type of joint resembling that of *Didelphys*. *Pantolambda* had well developed ridges on the radius and ulna indicating the presence of a strong interosseous ligament. In the development of man Gallois & Cade (1903) found that the ligament appeared at the third month, at the same time as other intermuscular septa, and though this evidence was not conclusive, it suggested that the ligament was a muscular neomorph, rather than, as had been previously supposed, a remnant of the skeletal system.

*Cebus capucinus.* In the Lemures and Anthropoidea the forearm bones have become bowed away from one another to increase the range of movement in pronation and supination to nearly two right angles, and the short strong interosseous ligament of typical mammals has become stretched to form a wide interosseous membrane. The bowing is found already fully developed in the Eocene *Notharctus* (Gregory, 1920), but is not developed in the tree shrew *Ptilocercus* (Clark, 1926). With the loss of the interosseous ligament as an important fulcrum the annular ligament has become very strong, and a powerful radio-ulnar ligament, eventually giving rise to the triangular fibrocartilage, has been developed at the wrist. The radial collateral ligament in *Cebus* has lost all attachment to the radius, so that it is inserted below on the annular ligament, and also directly on to the ulna. On the other hand, the epitrochleoradial ligament still forms a long, very oblique band passing from the medial epicondyle over the

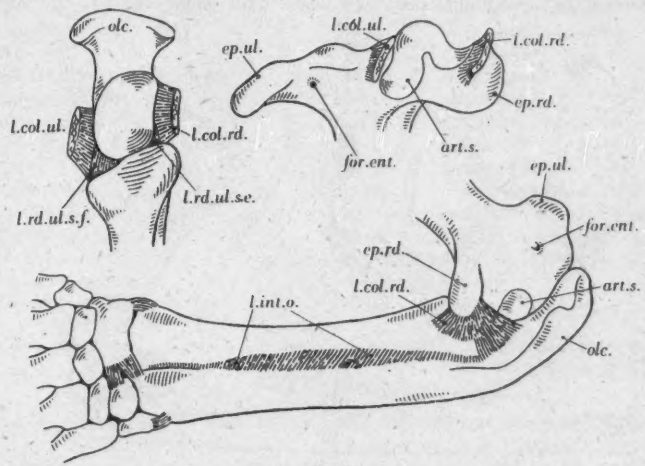


Fig. 8. *Echidna* sp.

coronoid process to the bicipital tuberosity of the radius.

This ligament is found in *Lemur*, *Hapale*, *Ateles*, and *Cercopithecus*, but is absent in *Papio* and *Macacus* and in the anthropoid apes (Hultkrantz, 1897; Parsons, 1900; Keith, 1894; Sullivan, 1933). It seems probable that in the ancestors of the primates both epitrochleo- and epicondylo-radial ligaments were developed as in typical mammals, but that with the increased range of pronation and supination in modern primates first the one and then the other ligament has been lost.

In my specimens of *Lemur*, *Nycticebus* and *Cebus* I have found that the ulna is capable of some rotation at the elbow joint during movements of pronation and supination, but that in *Macacus*, as in anthropoid apes, these movements are very small. In man, Hultkrantz (1897) found, by vivisection, that the movements amounted to only two

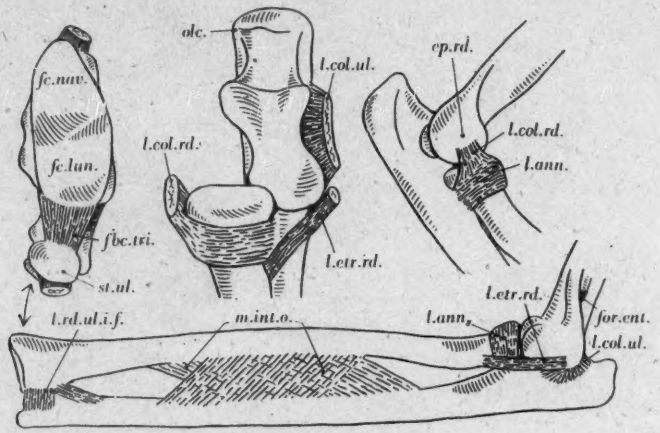


Fig. 9. *Cebus capucinus*.

or three degrees, but it will be understood that the human type of movement, in which the humero-ulnar joint is an almost perfect hinge, is extremely specialized.

*Muscles.* The evolution of the muscles of the forelimb has been revised recently by Howell (1936), Haines (1939) and Straus (1941, 1942). When the movements of any animal are known, the muscular work involved in their performance can be analysed; but, unfortunately, the nature and extent of these movements cannot be deduced with any certainty from a knowledge of the muscular arrangements, and must be worked out from a study of the articular surfaces and ligaments. The presence of strong pronator musculature in *Sphenodon*, *Varanus* and *Salamandra*, passing between the radius and ulna, indicates the existence of considerable relative movement between these bones, but the arrangement of the fibres cannot decide just what that movement is. In reptiles and amphibians the supinator manus, the representative of the abductor pollicis longus and the extensor pollicis brevis, is well placed for supination. Hultkrantz (1897) attributed the loss of the epitrochleo-anconeus in man to the absence of accessory movements of the ulna. Also he suggested that the anconeus had been retained as it had become an extensor, but it seems rather (Haines, 1939) that it has always been an extensor, though it may have had secondary functions.

## SUMMARY

1. In reptiles, both living and fossil, the humerus carries radial and ulnar condyles separated by an intercondylar groove. The radius and ulna articulate with each other at the superior radio-ulnar joint, but diverge towards the wrist, while their shafts are bowed away from each other, and there is no interosseous ligament.

2. Movements of flexion and extension occur at the elbow, and pronation and supination amount to about half a right angle. In pronation and supination, the rotation of the radius occurs chiefly at the elbow, while most of the ulnar rotation occurs at the wrist, although there is some rotation at both ends of each bone.

3. In urodeles there is an inferior radio-ulnar joint, but the movements of pronation and supination have remained unchanged.

4. In mammals the radius and ulna have become closely parallel to each other, and are bound together by a strong interosseous ligament along whose length the axis of the movements of pronation and supination now lies.

5. The monotremes are highly specialized forms,

and cannot be used to illustrate the commencement of a phylogenetic line leading to conditions in other mammals.

6. In primates the radius and ulna have become bowed away from one another so as to increase the range of pronation and supination, and the interosseous ligament has lost its original mechanical importance.

## KEY TO LETTERING

| | |
|---|---|
| *art.s.* | articular surface |
| *at.b.t.* | attachment to bicipital tuberosity |
| *at.e.r.* | attachment to extensor surface of radius |
| *cap.* | capitulum |
| *cnd.rd.* | radial condyle |
| *cnd.ul.* | ulnar condyle |
| *ep.rd.* | radial epicondyle |
| *ep.ul.* | ulnar epicondyle |
| *fbc.tri.* | triangular fibro-cartilage |
| *f.c.lab.* | fibro-cartilaginous labrum |
| *fc.lun.* | facet for lunate |
| *fc.nav.* | facet for navicular |
| *fc.ul.* | ulnar facet |
| *for.ect.* | ectepicondylar foramen |
| *for.ent.* | entepicondylar foramen |
| *gr.in.cnd.* | intercondylar groove |
| *gr.sty.* | groove for styloid process |
| *gr.ul.* | ulnar groove |
| *l.ann.* | annular ligament |
| *l.col.rd.* | radial collateral ligament |
| *l.col.ul.* | ulnar collateral ligament |
| *l.eco.co.* | epicondylo-coronoidal ligament |
| *l.eco.rd.* | epicondylo-radial ligament |
| *l.etr.an.* | epitrochleo-anconeal ligament |
| *l.etr.col.* | epitrochleo-coronoidal ligament |
| *l.etr.rd.* | epitrochleo-radial ligament |
| *l.ime.* | intermediate ligament |
| *l.int.o.* | interosseous ligament |
| *l.ps.cp.* | piso-carpal ligament |
| *l.ps.ul.* | piso-ulnar ligament |
| *l.rd.ul.i.e.* | inferior radio-ulnar ligament (extensor surface) |
| *l.rd.ul.i.f.* | inferior radio-ulnar ligament (flexor surface) |
| *l.rd.ul.s.e.* | superior radio-ulnar ligament (extensor surface) |
| *l.rd.ul.s.f.* | superior radio-ulnar ligament (flexor surface) |
| *l.ul.cp.* | ulno-carpal ligament |
| *m.int.o.* | interosseous membrane |
| *olc.* | olecranon |
| *pat.ul.* | ulnar patella |
| *pis.* | pisiform |
| *prj.rd.* | projection from radius |
| *rle.* | radiale |
| *rd.fl.* | flange on radius |
| *rg.cap.* | ridge separating capitulum |
| *rg.in.cnd.* | ridge engaging with intercondylar groove |
| *rg.ul.* | ridge engaging with ulnar groove |
| *troch.* | trochlear surface |
| *ule.* | ulnare |
| *ul.fl.* | flange on ulnar |

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# SYMPATHETIC RAMI IN MAN

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In a previous communication (Sheehan & Pick, 1943) we reported on the connexions and constitution of the rami communicantes in the rhesus monkey. Four general types of sympathetic branches were differentiated according to their fibre content: Type I representing the white rami, Types IIA and IIB, two varieties of grey rami differing from each other according to the number of fine medullated fibres contained within each, and Type III which included the truly mixed rami. The fine myelinated fibres (under 3 $\mu$ in diameter), which were present to some extent in all grey rami, occurred in particularly large numbers in the grey rami of C8 and T1 and of L6 and L7 spinal nerves. Their abundance in these regions, namely, above and below the thoraco-lumbar outflow, attracted our special attention, and it seemed important to find out whether a similar arrangement existed in man.

A review of the literature revealed that, although the topographical anatomy of the human sympathetic chain had been frequently studied (more recently in the cervical region by Axford (1928), Siwe (1931), Laubmann (1931), Sheehan (1933), Kirgis & Kuntz (1942), and in the lumbo-sacral region by Romankevič (1930), Labbok (1932, 1937), Trumble (1934), Fagarasanu (1938), yet there were few histological investigations reported, none of which were complete. The first, and still the best, study of the constitution of the human sympathetic rami, following the discovery of the thoraco-lumbar outflow, was made by Harman (1898–1900). Using the osmic acid stain on teased preparations he examined only the rami of C5 to T4 and of T11 to L4 inclusively. In the rami of C5, C6, C7 and C8, and again of L3 and L4 (i.e. above and below the levels of the sympathetic outflow) he observed myelinated fibres 'under 4 $\mu$ in diameter', but never more than 30 at any one spinal level. It is, of course, difficult to make accurate counts of medullated fibres in teased preparations, and even Harman's enumeration of the small myelinated fibres in the rami of the thoraco-lumbar outflow is far below what we now know to be present. It is curious that Harman does not specifically differentiate between grey and white rami in the thoraco-lumbar region.

The histological appearance of the human sympathetic rami received only brief mention in the account by Müller (1909), although he evidently made Weigert-stained preparations of many of the spinal nerves and rami communicantes. His illustrations, though clearly demonstrating the existence of white, grey and mixed rami, give no indication of their constitution at different segmental levels. The rami of C8 and T1 in man were specially examined by Johnson & Mason (1921), but they were primarily interested in determining which was the uppermost white ramus and not in the appearance of the grey rami. Kiss & Mihálik (1929) from quite an extensive study, having arrived at the conclusion that there was no clear distinction between white and grey rami (both containing myelinated and unmyelinated fibres), did not proceed to give any description of the constitution of the various rami, and the first, and apparently the only, complete series of sympathetic branches examined in man was that reported by Juba (1930) from Weigert stained material. The wide distribution of medullated fibres, many of the smallest calibre, within the rami of the cervical and lower lumbar nerves was very evident. Although detail regarding each ramus is lacking from the text, it is clear from the illustrations that the ramus to S1, for example, contains several hundred fine myelinated fibres. The rami from the upper cervical region are sketched to show comparable numbers of medullated fibres, but here they are evidently of much larger size.

Botár (1932), Woollard & Norrish (1933), and others, have examined at various times the constitution of some of the sympathetic branches in man, but there is lacking from the entire literature any complete account of the rami at all levels, as we have for the monkey and lower animals (dog, cat, rabbit).

## MATERIAL AND METHODS

Specimens of human rami, adequate for detailed histological study are, of course, not easily obtainable. For purposes of identifying each ramus a long and tedious dissection is essential and this is obviously difficult to undertake following a routine autopsy, and dissecting room material, even from still-births, is generally unsatisfactory for subsequent microscopical study of the nervous system. We were fortunate to obtain two cadavers injected intravascularly with 10% formol-saline shortly after death. Complete dissections with the aid of magnification were made in one specimen of the entire sympathetic chain and all its branches on both sides, in the other the same as far down as the tenth thoracic ganglion (below this level dissection was impossible). The dissections were then drawn diagrammatically, but in complete detail and every

ramus labelled by number. Subsequently, all sympathetic branches were removed and placed in 10 % formol-saline for 24 hr., washed and stained in bulk in 1 % osmic acid, and sectioned in paraffin. This material was supplemented by other examples of particular rami, mostly from the upper and lower levels of the thoraco-lumbar outflow, which were removed from eight other subjects where post-mortem examination was undertaken a few hours after death. Careful identification of such rami was made in every instance.

In order to have as a guide a basic plan of the number and distribution of the rami and of the commoner variations encountered, 25 other dissections were completed and sketched, each of an entire sympathetic chain together with all its rami to spinal nerves. All except two of these were made on foetal material. One foetus was anencephalic. The statistical data concerning general topography have all been compiled from this series of twenty-five dissections.

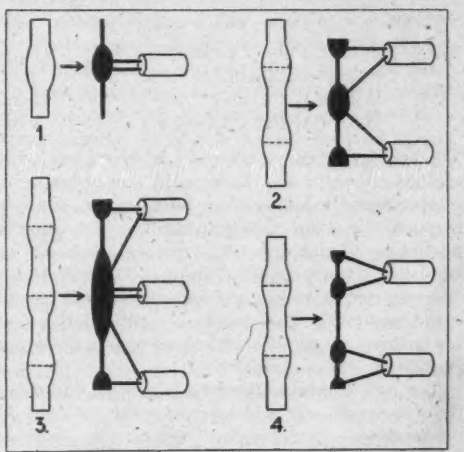
## GENERAL TOPOGRAPHY AND NOMENCLATURE

Irregularity in the number of ganglia along the sympathetic chain is a familiar observation. Anatomical texts refer to 'ten or eleven' thoracic ganglia, 'four up to eight' lumbar, and 'three or four' sacral ganglia, 'though fusion with neighbouring ganglia may reduce the number still further'. This does not, however, tell the full story, for the process of fusion is not simply a combination of adjacent ganglia. Certainly in the lower thoracic and in the lumbar region, where irregularity is even more marked in man than in the monkey, there is, if we have interpreted the facts correctly (Sheehan & Pick, 1943), division of each primordial ganglionic mass* into cranial and caudal portions, the ultimate fate of which will determine the number and the type of ganglia to be found at that particular segmental level (Text-fig. 1):

(1) The two portions may fuse together again (or never really separate) producing a single ganglion with connexions to only one spinal nerve, a type of ganglion truly segmental and most commonly seen in man in the upper thoracic region.

(2) A second possibility would be the fusion of the caudal half of one primordial mass with the cranial half of the next lower, forming a ganglion with connexions to two spinal nerves; therefore not strictly segmental and found typically in the lower thoracic region in man.

(3) A third possibility would be a variation of the second, namely a fusion of portions of more than two primordial masses. All combinations are possible and therefore all types of ganglia with connexions to three or more spinal nerves. This arrangement is represented typically by the superior and inferior cervical ganglia and occurs quite commonly in the lumbar region in man.




Text-fig. 1. Schema illustrating the development of possible variations of the ganglionic chain from the primordial cell column.

(4) A fourth arrangement would result from one or both of the portions of the primordial mass persisting as a separate ganglion, thus producing additional ganglia or complete duplication.

(5) Lastly a primordial mass, or a portion of it, may appear to be missing altogether, when the rami usually connected with it come off the sympathetic chain directly.

All these varieties can therefore be recognized if the ganglia are numbered in accordance with the distribution of the rami. Indeed it is the only method of determining the proper enumeration of the ganglia and of making a true comparison between several dissections. Emphasis has been laid on this point since the confident statements in surgical literature regarding the removal of the

* According to Kuntz (1920) the primordia of the sympathetic trunks arise in human embryos about 5 mm. in length as small groups of cells lying segmentally along the dorso-lateral aspects of the aorta in the lower thoracic and upper abdominal regions. In embryos 9–10 mm. in length the sympathetic primordia are present from the upper cervical to the sacral regions, but due to the curvature of the embryo they are in such close proximity to each other as to appear as a continuous column of loosely aggregated cells. Secondary segmentation occurs at a later stage. In the cervical region there occurs a gradual extension cephalad of the cells constituting the sympathetic primordia of the upper thoracic region, but whether each cervical segment makes its contribution of cells to these primordia is still uncertain. There appears to be no account of the development specifically of the lumbo-sacral portion of the sympathetic chain. A further study of the ganglion crests of this region in relation to the sympathetic primordia is needed.

'third' or the 'fourth' lumbar ganglion are unintentionally misleading. To designate arbitrarily, as some have done, a ganglion lying on the body of the third lumbar vertebra, for example, as the 'third lumbar' ganglion, has no merit, for in the next case a similarly placed ganglion may bear no resemblance whatever in the distribution of its rami and indeed may have no connexion at all with the third lumbar spinal nerve.

A detailed account of the topographical anatomy of the human sympathetic chain need not be recorded here, but with the schema of development (outlined above) in mind, the following brief analysis of the ganglia and rami has been made for purposes of orientation (see Pl. 1). The *superior cervical ganglion*, large and fusiform, extended in every instance from the base of the skull to the level of the upper (or lower) border of the second cervical vertebra.

| | | |
|---|---|---|
| Rami*: | With C1 and C2 (incl. loops of) (cervical plexus) | 11 inst. |
| | With C1, C2, and C3 | 11 inst. |
| | With C1, C2, C3, and C4 | 3 inst. |

The upper two cervical nerves received two, three and occasionally four rami each. The ramus to C3 often formed a loop with a lower ramus from the sympathetic chain, and from this loop fine branches passed to C3 and sometimes to C4. In almost half the cases C3 was omitted from the distribution of the superior cervical ganglion and formed its only connexion with the cervical sympathetic chain lower down; twice it received no apparent sympathetic connexion at all.

The *middle cervical ganglion* was very variable in its occurrence, size and form; sometimes single, in others double, sometimes of medium size, in others only represented by a tiny ganglionic knot. In one example (no. 13L), an anencephalic foetus, the entire cervical sympathetic chain consisted of a fused ganglionic mass uniting the superior and inferior cervical ganglia:

| | | |
|---|---|---|
| Occurrence: | | |
| Absent | | 5 inst. |
| Single | | 10 inst. (incl. no. 13L). |
| Double | | 10 inst. |
| Position: | | |
| When single: | *either* 'High' position (C5) | 7 inst. |
| | *or* 'Low' position (C7) (on posterior loop of ansa†) | 2 ,, |
| When double: | *either* Both above ansa (C5) | 3 inst. |
| | *or* One above ansa (C5), one on posterior loop of ansa† (C7) | 7 ,, |

* Only the rami communicating with spinal nerves are here considered.

† Sometimes referred to as the 'vertebral' ganglion and included as part of stellate configuration.

Rami:
When single: to C4, C5 and C6 (occasionally C3).
When double: upper ganglion—to C4 and C5.
lower ganglion—to C6 (occasionally C7).

There were often two or three rami to each spinal nerve and frequent interconnexions and loops between the rami. Some of the sympathetic branches, particularly those to C4 and C5, pierced the prevertebral muscles to reach the spinal nerve (designated as 'ramus profundus' by Wrete, 1934).

The *inferior cervical ganglion* was fusiform or stellate in shape and in many examples was partially or completely fused with the first, and occasionally second, thoracic ganglia.

| | | |
|---|---|---|
| Occurrence: | | |
| Independent | | 5 inst. |
| Fused with first thoracic ganglion | | 17 ,, |
| Fused with first and second thoracic ganglia | | 3 ,, |
| Position: | | |
| At level of intervertebral disc C7–T1. | | |
| When fused with first thoracic—extended down to intervertebral disc T1–T2. | | |
| When fused with first and second thoracic—extended down to T2 vertebral body (lower border). | | |
| Rami: | | |
| Where independent—to C6, C7 and C8 (occasionally C5). | | |
| When fused with first thoracic—to T1 and T2 in addition. | | |
| When fused with first and second thoracic—to T3 in addition. | | |

The number of rami to each cervical spinal nerve was very variable, there being 2, 3 or 4 branches to each nerve, and frequently these arose from a common trunk e.g. the 'vertebral' nerve. (For details of arrangement see Sheehan, 1933.) The first thoracic nerve invariably received two and often three short stout rami, one of which entered the nerve medial, the others lateral, to the ganglion. They were designated as 'proximal' and 'distal' according to their points of junction with the spinal nerve, relative to one another. The contribution from T2 to the brachial plexus almost invariably received a special ramus from the lower part of the 'stellate' ganglion.

The *first thoracic ganglion* was only distinguishable as an independent ganglion in five instances. As such it was of medium size and was placed opposite the body of the first thoracic vertebra or the intervertebral disc T1–T2. Its rami were usually three short stout branches to T1 and a fine single branch down to T2, which often joined the contribution from that nerve to the brachial plexus.

The *second thoracic ganglion* was independent of the stellate in 22 instances although in 7 of these

examples the connecting sympathetic chain was almost as thick as the ganglion itself. It was placed opposite the lower border of T2 vertebral body or the intervertebral disc T2–T3. Its rami were two (occasionally 3) stout branches to T2 and in 4 instances an additional slender branch to T3.

The *thoracic ganglia 3rd–10th showed a regular* segmental pattern, though some partial fusion or merging of individual ganglia occurred in a few instances, e.g.

| | | | |
|---|---|---|---|
| Between 3rd and 4th thoracic | | | 3 inst. |
| ,, | 4th and 5th | ,, | 5 ,, |
| ,, | 5th and 6th | ,, | 1 ,, |
| ,, | 6th and 7th | ,, | 1 ,, |
| ,, | 7th and 8th | ,, | 4 ,, |
| ,, | 8th and 9th | ,, | 2 ,, |
| ,, | 9th and 10th | ,, | 2 ,, |

(In two examples, no. 13R and no. 13L, there was almost one ganglionic mass between the 3rd and 7th thoracic.) Even where such merging existed the individual segmental ganglia were easily distinguishable. They were of medium size, becoming distinctly larger below the 5th or 6th thoracic, i.e. at the levels of exit (and entry) of the splanchnic nerve fibres.

Position: opposite intervertebral disc between corresponding and next lower vertebrae, or at the level of the upper border of next lower vertebra, e.g. 5th thoracic ganglion at level of intervertebral disc T5–T6 or at upper border of T6 vertebral body. Thus the thoracic ganglia were placed slightly lower than the corresponding nerve and the rami, short and relatively stout, passed obliquely upwards from the ganglion.

Rami: Two or three (rarely four) short branches* to the corresponding nerve, one usually travelling upwards and medially, the others upwards and laterally, from the ganglion.

Each ganglion occasionally supplied an additional ramus to the next lower spinal nerve, such an arrangement occurring in the following frequency:

| | | | |
|---|---|---|---|
| At 3rd thoracic ganglia | | | 2 inst. |
| ,, 4th | ,, | ,, | 2 ,, |
| ,, 5th | ,, | ,, | 0 ,, |
| ,, 6th | ,, | ,, | 3 ,, |
| ,, 7th | ,, | ,, | 3 ,, |
| ,, 8th | ,, | ,, | 4 ,, |
| ,, 9th | ,, | ,, | 5 ,, |
| ,, 10th | ,, | ,, | 14 ,, |

Out of a total of only twenty-five dissections these numbers have not in themselves any special significance, but the increasing frequency towards the lower end of the thoracic chain has a definite meaning, for the pattern of ganglion is the one typically found in the upper lumbar region.

The *last thoracic ganglia*. There were one or two thoracic ganglia below the tenth, according to the form of splitting and subsequent fusion which takes place in the primordial ganglionic mass. Thus in 7 instances there was a small ganglion connected only with T11 spinal nerve, and in 4 others there was a small ganglion connected only with T12 spinal nerve. These small 'extra' ganglia were present in addition to a larger ganglion connected with both T11 and T12 and designated here simply as the 'last thoracic'. It was usually placed on the body of the twelfth thoracic vertebra and was sometimes fused with the ganglion above (3 inst.) or below (4 inst.) or both (3 inst.). It was only present therefore as an independent ganglion in a little over half the specimens studied.

The *lumbar ganglia* in man were so irregular that identification and enumeration were often very difficult, much more so than in the monkey. Such wide fusion occurred in some instances that the greater part of the lumbar chain consisted of a single elongated ganglionic mass. It would be of little value to record that the number of ganglia in the lumbar region varied from two to six, with four or five occurring in over three-quarters of the cases. The type of fusion is so different from case to case that two examples with four ganglia each may bear no resemblance. Anatomically the soundest way is to break down the various fused chains of ganglia which occur in the lumbar region into their component primordial masses, according to their communicating branches, but it will be readily seen that such a plan would be of little service to the surgeon. We have therefore enumerated the lumbar and sacral ganglia according to the pattern followed in the previous study on the monkey (Sheehan & Pick, 1943), since also in man it reveals the commonest type of ganglion found independently at most levels. The frequency of massive fusion, however, must place on guard the surgeon seeking for any specific lumbar ganglion. With this fully in mind, the following analysis of the lumbar and sacral ganglia has been made. The statistics from such a small series (a total of twenty-five dissections) have only relative merit, but serve to indicate general tendencies in one direction or another.

*First lumbar ganglion*, i.e. with connexions to T12 and L1 spinal nerves. Present as an independent ganglion in 13 instances and then placed on the first lumbar vertebra or the intervertebral disc below. In other examples it was recognizable but fused with the ganglion above (3 inst.) or below (4 inst.) or both (3 inst.), and was accordingly displaced. In the remaining two examples its component primordial parts were separate and remained as independent ganglia or were fused with the next adjoining ganglion; no examples, however,

* The variation in number of rami to each spinal nerve is not of great significance for they differed so greatly in thickness in different dissections, and splitting and joining of rami were so common.

were found of a ganglion connected only with the first lumbar nerve.

*Second lumbar ganglion*, i.e. with connexions to L1 and L2 spinal nerves. Present as an independent ganglion in 12 instances, and placed on the second lumbar vertebra or the intervertebral disc below. In other examples it was recognizable, but fused with the ganglion above (6 inst.), or below (1 inst.), and was accordingly displaced. It was apparently missing in 2 instances and in the remaining 4 examples its component primordial parts were 'split' as indicated above for the first lumbar ganglion; no examples, however, were found of a ganglion connected only with the second lumbar nerve.

*Third lumbar ganglion*, i.e. with connexions to L2 and L3 spinal nerves. Present as an independent ganglion in only 2 instances (situated on third lumbar vertebra), but distinguishable in many others, fused with the ganglion above (2 inst.), below (8 inst.) or both (7 inst.) and accordingly displaced. In the remaining 4 examples it was 'split' as indicated above for the first two lumbar ganglia, and 3 examples altogether were found of a ganglion connected only with the third lumbar nerve.

*Fourth lumbar ganglion*, i.e. with connexions to L3 and L4 spinal nerves (or only L4). Present as an independent ganglion connected with both nerves in only 1 instance (situated on fourth lumbar vertebra), but distinguishable in several others, fused with the ganglion above (2 inst.), below (6 inst.) or both (4 inst.) and accordingly displaced. In 12 examples (almost half of the cases) its component primordial parts were separate and remained as independent ganglia or were fused with the next adjoining ganglion. Thus 11 examples were found of a ganglion connected only with the fourth lumbar nerve, and this must be considered its commonest form.

*Fifth lumbar ganglion*, i.e. with connexions to L4 and L5 spinal nerves (or only L5). Present as an independent ganglion connected with both nerves in only 4 instances (situated on fifth lumbar vertebra), but distinguishable in a few others, fused with the ganglion above (1 inst.), below (1 inst.) or both (1 inst.). In 18 examples its component primordial parts were separate and remained as independent ganglia or were fused with the next adjoining ganglion; altogether 15 examples occurred of a ganglion connected only with the fifth lumbar nerve, obviously the commonest form at this level. Such examples (like those of the fourth lumbar) differ from the truly segmental pattern seen in the thoracic region for they usually represent only one part of the original segmental primordial mass.

The *sacral ganglia*, with the exception sometimes of the first, were very much smaller than any others in the sympathetic chain. On the whole they tended to follow the pattern described for the lumbar ganglia, but there was an increasing frequency for the occurrence of a ganglion connected by a single ramus with only one spinal nerve.

*First sacral ganglion:*
Connected with L5 and S1 spinal nerves 13 inst.
(in 5 of these examples, fusion above or below)
Connected with S1 alone 12 inst.

*Second sacral ganglion:*
Connected with S1 and S2 spinal nerves 14 inst.
(in 5 of these examples, fusion above or below)
Connected with S2 alone 11 inst.

*Third sacral ganglion:*
Connected with S2 and S3 spinal nerves 13 inst.
(in 3 of these examples, fusion above or below)
Connected with S3 alone 12 inst.

*Fourth sacral ganglion* (missing altogether once):
Connected with S3 and S4 spinal nerves 17 inst.
(in 16 of these examples, fusion above or below)
Connected with S4 alone 7 inst.

*Fifth sacral ganglion* (missing altogether 6 times):
Connected with S4 and S5 spinal nerves 13 inst.
(in 10 of these examples, fusion above or below)
Connected with S5 alone 6 inst.

*Ganglion Impar.* Out of 12 cadavers, it was present 9 times: 6 times in the midline, 3 times at the end of the right chain. It distributed fine branches to the coccygeal nerves.

*Intermediary ganglia*, namely, ganglia placed along the course of a ramus, were surprisingly common, occurring somewhere along the sympathetic chain in practically every dissection. They were most frequently found in the cervical and lower lumbar regions, in the former quite small, but in the latter attaining the size of and sometimes replacing the segmental ganglion of the chain. The frequency at various levels is worth recording, since with such an arrangement lumbar sympathectomy as generally performed would leave these ganglia intact. Intermediary ganglia were found:

| | | |
|---|---|---|
| On ramus to | C4 | 4 inst. |
| ,, | C5 | 1 ,, |
| ,, | C6 | 2 ,, |
| ,, | T10 | 1 ,, |
| ,, | T12 | 1 ,, |
| ,, | L1 | 1 ,, |
| ,, | L2 | 1 ,, |
| ,, | L3 | 4 ,, |
| ,, | L4 | 6 ,, |
| ,, | L5 | 8 ,, |
| ,, | S2 | 1 ,, |

A *double* (or occasionally triple) *sympathetic chain*, though extremely common and occurring anywhere in the thoracic, lumbar or sacral regions, apparently does not present any common surgical problem for it never extended in any of the dissections for more

than the distance between two adjacent ganglia. This was true also in the monkey. *Transverse interconnexions* between the sympathetic chain of one side with that of the other, are equally of little if any surgical significance, for in this study they never occurred above the 5th lumbar level and were almost entirely confined to the terminations of the chains over the ventral surface of the sacrum. They were here quite common (in 9 out of 12 cadavers) and formed thin connecting branches between the ganglia or chains of each side, just above the ganglion impar. The illustrations given in some older texts of rich anastomoses between the two sympathetic chains all the way down, we believe to be erroneous.

The *origin of the splanchnic nerves* from the sympathetic chain was noted in most of the dissections; the uppermost branch emerged:

| | | | |
|---|---|---|---|
| From | 4th | thoracic ganglia | 1 inst. |
| ,, | 5th | ,, ,, | 2 ,, |
| ,, | 6th | ,, ,, | 11 ,, |
| ,, | 7th | ,, ,, | 7 ,, |
| ,, | 8th | ,, ,, | 4 ,, |

Below the twelfth thoracic vertebra, similar branches known as the 'lumbar splanchnics' passed also to the coeliac plexus. These left the sympathetic chain at many points as far down as the third lumbar vertebra in almost every example.

## CONSTITUTION OF RAMI

Classification of the human rami, according to their fibre constitution, into the four types already described in the monkey (Sheehan & Pick, 1943), was such an obvious and relatively simple process that the validity of the types was more than ever apparent. The two varieties of grey rami (Pl. 2, figs. *c*, *d*) stand out in sharp contrast with the white and mixed rami (Pl. 2, figs. *a*, *e*), giving no support whatever to the contention of Kiss and Mihálik (1929) that the rami cannot be differentiated histologically. In man, as in the monkey, the white rami (type I) consisted of myelinated fibres of all sizes, ranging up to 15–20 $\mu$ in diameter, and the smallest fibres (3 $\mu$ and less) by no means dominated the picture. Again, the mixed rami (type III) showed a single nerve bundle containing areas typical of type I and others resembling closely the grey rami of type IIA.

The analysis, according to fibre constitution, of the various rami of each spinal nerve, has been condensed in Tables 1–3, in which, for the sake of clarity the letter '*w*' stands for type I (white ramus), '*m*' for type III (mixed ramus), '*g*' for type IIA (characteristic grey ramus), and '*G*' for type IIB (grey ramus heavily 'peppered' throughout with small medullated fibres under 3 $\mu$ in diameter and several hundred in number).

The limits of the thoraco-lumbar outflow can be seen at a glance at Tables 1 and 3. In Table 1 the upper level has been defined in six instances at T1. Mixed rami appear for the first time in the branches connected with this nerve, and typical white rami in those connected with T2. In two examples (PII.R and PII.L) one of the distal rami to C8 showed the appearance of type IIA grey ramus with the characteristic area of ten to twenty large and medium myelinated fibres (10 to 5 $\mu$ in diameter) near its circumference, but in this area, which was relatively insignificant in extent, a number of fine medullated fibres under 3 $\mu$ in diameter could be seen. It appeared therefore as a possibility that this represented a mixed ramus (type III) connected with the eighth cervical nerve, which would constitute a higher thoraco-lumbar outflow in this one case (the dissections were from the right and left sides of the same subject). In Table 3 the lower level of the thoraco-lumbar outflow has been defined in all seven instances at L2. This definition of the outflow (T1–L2) from a microscopical study of the rami confirms therefore the results obtained from examination of the ventral spinal roots in man (Sheehan, 1941).

An observation made in the monkey, that the white ramus to T1 is that which joins the nerve most distally, has been confirmed in man. Table 2 reveals that this is no exception, but the rule, for most of the rami of the thoracic region. This is all the more surprising since the diagrams in every standard textbook show the opposite arrangement. In the lower thoracic and upper lumbar region the spinal nerves are connected generally by at least two rami, an upper one running transversely or descending slightly from the ganglion of the corresponding segment, and a lower one passing obliquely between the nerve and the ganglion of the next lower segment. Botár's observation (1932) that the 'transverse' rami are grey and the 'oblique' rami are white is amply confirmed by the findings recorded in Tables 2 and 3 where there are only two clear reversals of this arrangement.

The frequent occurrence of type IIB (indicated by '*G*' in Tables 1 and 3) in the rami of C7, C8 and in the vertebral nerve and again in the rami of L4, L5 and S1 signifies the relative abundance at these levels of fine myelinated fibres (under 3 $\mu$ in diameter). The fact that a similar distribution of this type of grey ramus exists in the cat and monkey, indicates some fundamental pattern, but what its significance may be is not yet clear. The nerves in question are distributed particularly to the blood-vessels and sweat glands in the hand and foot, which are notoriously rich in their sympathetic innervation.

The rami marked with an asterisk in Table 1 contain relatively large areas, and in some cases separate bundles, of large and medium-sized myelinated fibres (10–5 $\mu$ in diameter). It will be

**Table 1.** *Types of rami communicantes of cervical and upper thoracic spinal nerves*

| Dissection catalogue no. | Spinal nerves: C1 all rami | C2 all rami | C3 all rami | C4 all rami | C5 all rami | C6 all rami | C7 Prox. | C7 Dist. | Verteb. nerve | C8 Prox. | C8 Dist. | T1 Prox. | T1 Dist. | T2 From Stellate | T2 Prox. | T2 Dist. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PI.R. | *g* | *g* | — | *g* | *g** | *g** | *g* | *G* | *G* | *G* | *G* | *g* | *m* | *m* | *g* | *m* |
| | | | | | | | | | | | *g* | *g* | *m* | *w* | | *w* |
| PI.L. | *g** | *g** | *g** | *g* | *g** | *g** | *G* | *G* | *G* | *G* | *G* | *g* | *m* | *m* | *g* | *w* |
| | | | | | | | | | | | *g* | *g* | *g* | | | *m* |
| PII.R. | *g** | *g** | — | *g** | *g** | *g** | *g* | *g* | *g* | *g* | *g* ? *m* | *g* | *m* | — | *g* | *m* |
| | | | | | | | | | | | | *g* | *g* | | | |
| PII.L. | — | *g* | *g* | *g* | *g* | *g** | *g* | *g** | *g* | *g* | *g* ? *m* | *m* | *m* | *w* | *g* | *m* |
| | | | | | | | | | | | | *g* | *g* | | | |
| E.R. | — | — | — | — | — | — | *G* | *G* | *G* | *G* | *G* | *G* | *m* | *w* | *g* | *w* |
| | | | | | | | | | | | *g* | | | | | |
| G.R. | — | — | — | *g* | *g* | — | — | — | *g* | *g* | *g* | *G* | *m* | — | — | — |

*w* = Type I (white ramus). *m* = Type III (mixed ramus). *g* = Type II A (grey ramus). *G* = Type II B (grey ramus).
Where two (or more) separate nerve bundles in any ramus or two parallel rami—the constitution of both is indicated.

**Table 2.** *Types of rami communicantes of thoracic spinal nerves*

| Dissection catalogue no. | Spinal nerves: T3 Prox. | T3 Dist. | T4 Prox. | T4 Dist. | T5 Prox. | T5 Dist. | T6 Prox. | T6 Dist. | T7 Prox. | T7 Dist. | T8 Prox. | T8 Dist. | T9 Prox. | T9 Dist. | T10 Transv. from T9 gangl. | T10 Obliq. from T10 gangl. | T11 Transv. from T10 gangl. | T11 Obliq. from last T. gangl. | T12 Transv. from last T. gangl. | T12 Obliq. from L1 gangl. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PI.R. | *m* | *w* | *g* | *m* | *g* | *w* | *g* | *w* | *g* | *w* | *g* | *w* | *g* | *m* | *g* | *m* | *w* | *w* | *g* | *w* |
| | | | | *g* | | *g* | | *g* | | | | | | | | | *g* | *m* | | |
| PI.L | *g* | *w* | *g* | *m* | *g* | *w* | *g* | *w* | *g* | *w* | *g* | *m* | *g* | *w* | *g* | *w* | *g* | *w* | *w* | *w* |
| | | | | *g* | | *g* | | | | | | | | | | *m* | | *m* | | |
| PII.R. | *g* | *m* | *g* | *m* | *g* | *w* | *g* | *w* | *g* | *m* | *g* | *w* | *g* | *w* | *g* | *m* | — | — | — | — |
| | | *g* | | | | | | *g* | | | | *g* | | | | | | | | |
| PII.L. | *g* | *m* | *g* | *m* | — | *m* | *g* | *m* | *g* | *w* | *g* | *w* | *g* | *w* | *g* | *w* | — | — | — | — |
| | | *g* | | | | | | | | | | | | | | *g* | | | | |
| K.R. | — | — | — | — | — | — | — | — | — | — | *g* | *w* | *g* | *w* | *g* | *m* | *g* | *w* | *w* | *w* |
| | | | | | | | | | | | | | | | | *g* | | *m* | *g* | |

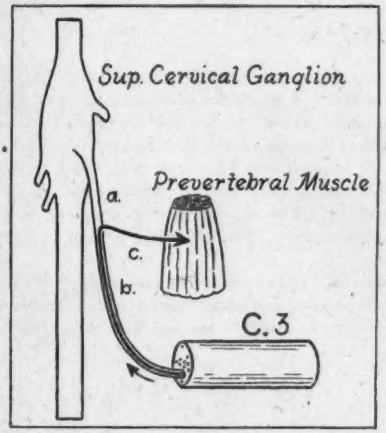
*w* = Type I (white ramus). *m* = Type III (mixed ramus). *g* = Type II A (grey ramus). *G* = Type II B (grey ramus).
Where two (or more) separate nerve bundles in any ramus or two parallel rami—the constitution of both is indicated.

Table 3. *Types of rami communicantes of lumbar and sacral spinal nerves*

| | Spinal nerves | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | L1 | | L2 | | | | | | | | | |
| Dissection catalogue no. | Transv. as from L1 gangl. | Obliq. as from L2 gangl. | Transv. as from L2 gangl. | Obliq. as from L3 gangl. | L3 all rami | L4 all rami | L5 all rami | S1 all rami | S2 all rami | S3 all rami | S4 all rami | S5 all rami |
| PI.R. | *g* | *w*<br>*w*<br>*w* | *g*<br>*g* | *w* | *g* | *g* | *G* | *G* | *g* | *g* | *g* | *g* |
| PI.L | — | *w* | *g* | *m* | *g* | *g* | *g* | *G* | *g* | *g* | *g* | — |
| B.R. | — | — | *g* | *m* | *g* | *g* | *G* | — | — | — | — | — |
| T.R. | *g* | *w* | *m*<br>*g* | *g* | *g* | *G* | — | — | — | — | — | — |
| S.R. | *g* | *w*<br>*m* | *m* | *g* | *g* | *g* | *G* | — | — | — | — | — |
| E.R. | *g* | *w*<br>*g* | *g* | *w*<br>*m* | *g* | *g* | — | — | — | — | — | — |
| K.R. | *g* | *m* | *g* | — | *g* | *g* | *g* | — | — | — | — | — |

*w* = Type I (white ramus). *m* = Type III (mixed ramus). *g* = Type II A (grey ramus). *G* = Type II B (grey ramus). Where two (or more) separate nerve bundles in any ramus or two parallel rami—the constitution of both is indicated.

noticed that such rami occur exclusively in the cervical region well above the thoraco-lumbar outflow. The exact course of these myelinated fibres has been traced in several examples, as indicated in Text-fig. 2, and shows conclusively that they are in reality part of the somatic innervation to the




Text-fig. 2. Diagram showing a grey ramus of the cervical region containing somatic motor fibres in its distal part. The motor fibres from C3 join the grey ramus along *b* and leave it, to reach the muscle, along *c*. Part *a* is devoid of somatic fibres.

prevertebral muscles. It is of interest that they do not merely run alongside the fibres of the grey ramus, but in most instances the two become incorporated into the same nerve bundle.

The roots of the splanchnic nerves as they emerge from the sympathetic chain, both in the thoracic and in the upper lumbar region, have precisely the same appearance histologically as the white rami. This is to be expected since they are composed largely of preganglionic and afferent fibres.

## SUMMARY

1. A study of the connexions of the human sympathetic chain in 25 dissections has revealed the commonest types of ganglia found at each segmental level. There was great complexity in the lumbar region and it was rarely possible to define an independent 'third' or 'fourth' lumbar ganglion. The different types of ganglia found respectively in the cervical, thoracic, lumbar and sacral regions reveal a fundamental pattern, indicative of a splitting and secondary fusion of each primordial ganglionic mass. Ganglia placed along the course of a ramus were surprisingly common especially in the lower lumbar region, and resulted of course from a greater degree of peripheral migration of cells than usually obtains.

2. A double sympathetic chain occurred anywhere along the sympathetic chain but never extended for more than the distance between two adjacent ganglia and probably therefore does not present any surgical problem. Transverse connexions between the sympathetic chains of each side were never found above the level of the fifth lumbar vertebra. The possibility of any bilateral innervation of the limbs via such transverse connexions can be almost certainly excluded.

3. Examples of rami connected with every spinal nerve have been studied histologically. The same four types of rami, according to fibre constitution, as described previously in the monkey, are present in man. In the thoracic region it was the rule for



*w* = Type I (white ramus). *m* = Type III (mixed ramus). *g* = Type II A (grey ramus). *G* = Type II B (grey ramus). Where two (or more) separate nerve bundles in any ramus or two parallel rami—the constitution of both is indicated.

the white (or mixed) ramus to be that which joins the spinal nerve most distally; in the lower thoracic and upper lumbar regions the 'transverse' rami were almost invariably grey, and the 'oblique' white.

4. The number of fine myelinated fibres (under 3 $\mu$ in diameter) within grey rami has been greatly underestimated. They occurred in great numbers in the rami of C7 and C8 and again in those of L5 and S1, a distribution corresponding to that found in the cat and monkey.

5. The thoraco-lumbar outflow in man has been confirmed as T1–L2 (inclusive).

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## EXPLANATION OF PLATES

### PLATE 1

From a dissection of the sympathetic chain, rami communicantes and splanchnic nerves (adult human). The preganglionic rami are indicated in red. The postganglionic rami, the sympathetic chain and the splanchnic nerves are shown in black.

### PLATE 2

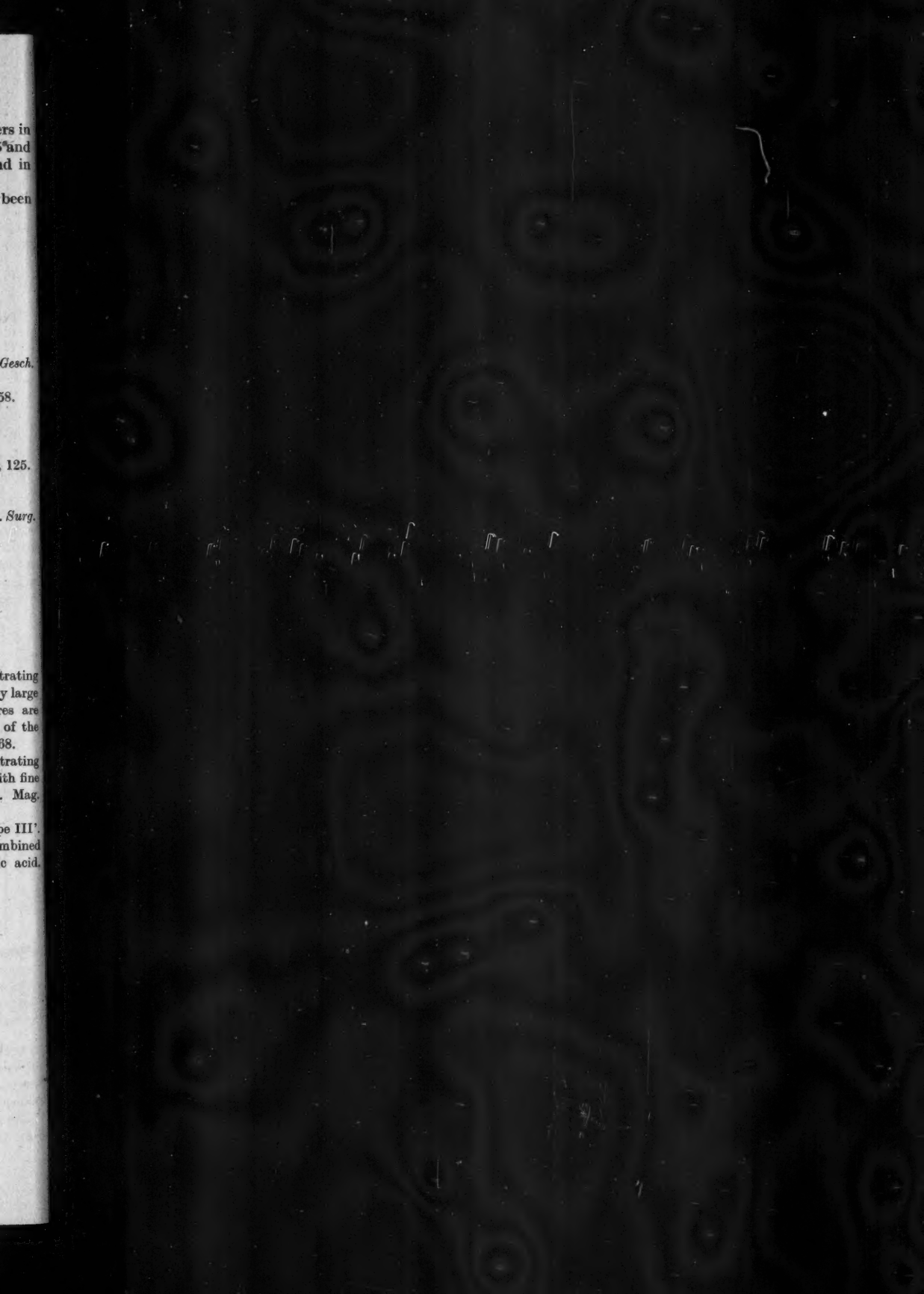
*a*. Section of a white ramus communicans representing 'Type I'. It contains myelinated fibres of all sizes. Stained with 1% osmic acid. Mag. ×185.

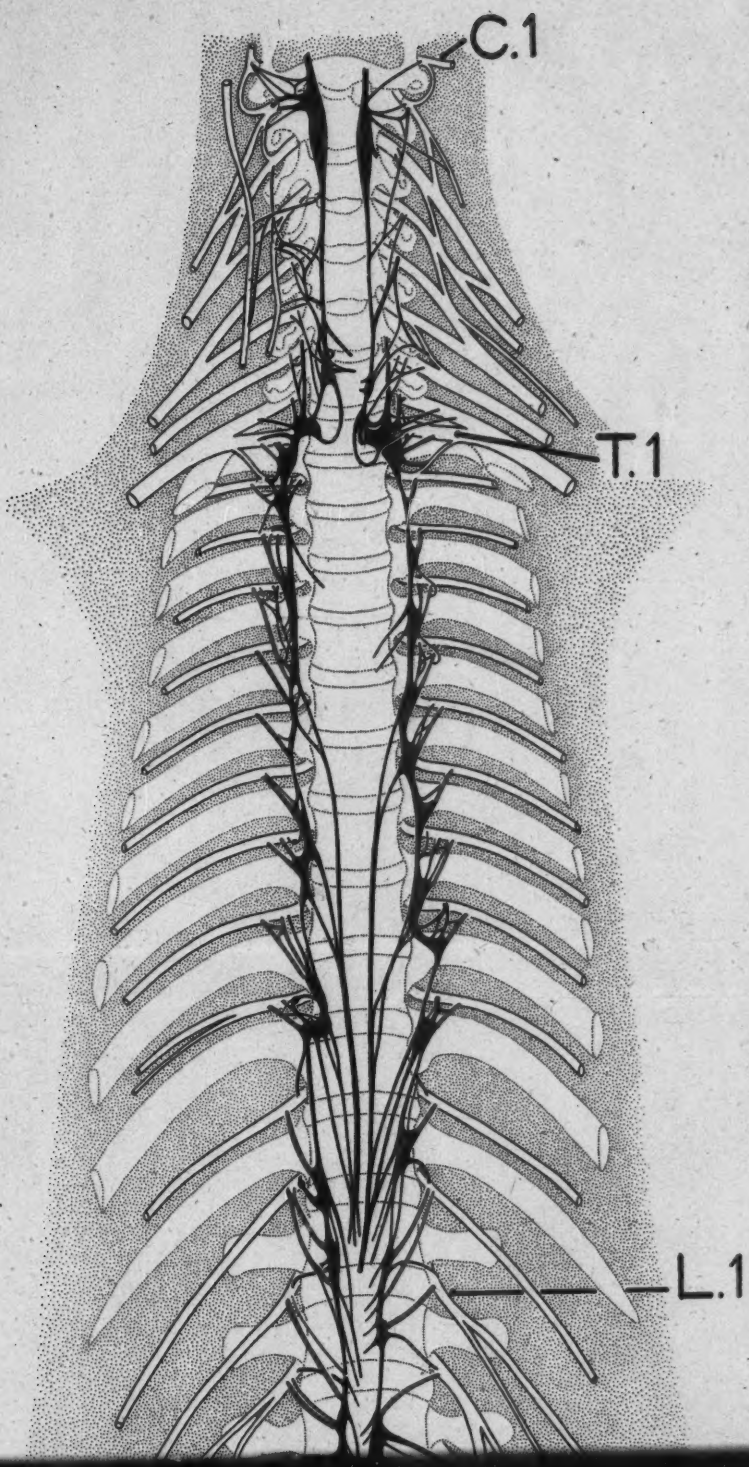
*b*. High-power view of the section shown in fig. *a*. Mag. ×556.

*c*. Section of a grey ramus communicans illustrating 'Type II A'. Note the two small areas occupied by large myelinated fibres. A few fine myelinated fibres are scattered throughout the unmyelinated portion of the nerve. Stained with 1% osmic acid. Mag. ×368.

*d*. Section of a grey ramus communicans illustrating 'Type II B'. The nerve is heavily 'peppered' with fine myelinated fibres. Stained with 1% osmic acid. Mag. ×185.

*e*. Section of a mixed ramus communicans of 'Type III'. The myelinated and unmyelinated portions are combined in one nerve bundle. Stained with 1% osmic acid. Mag. ×185.

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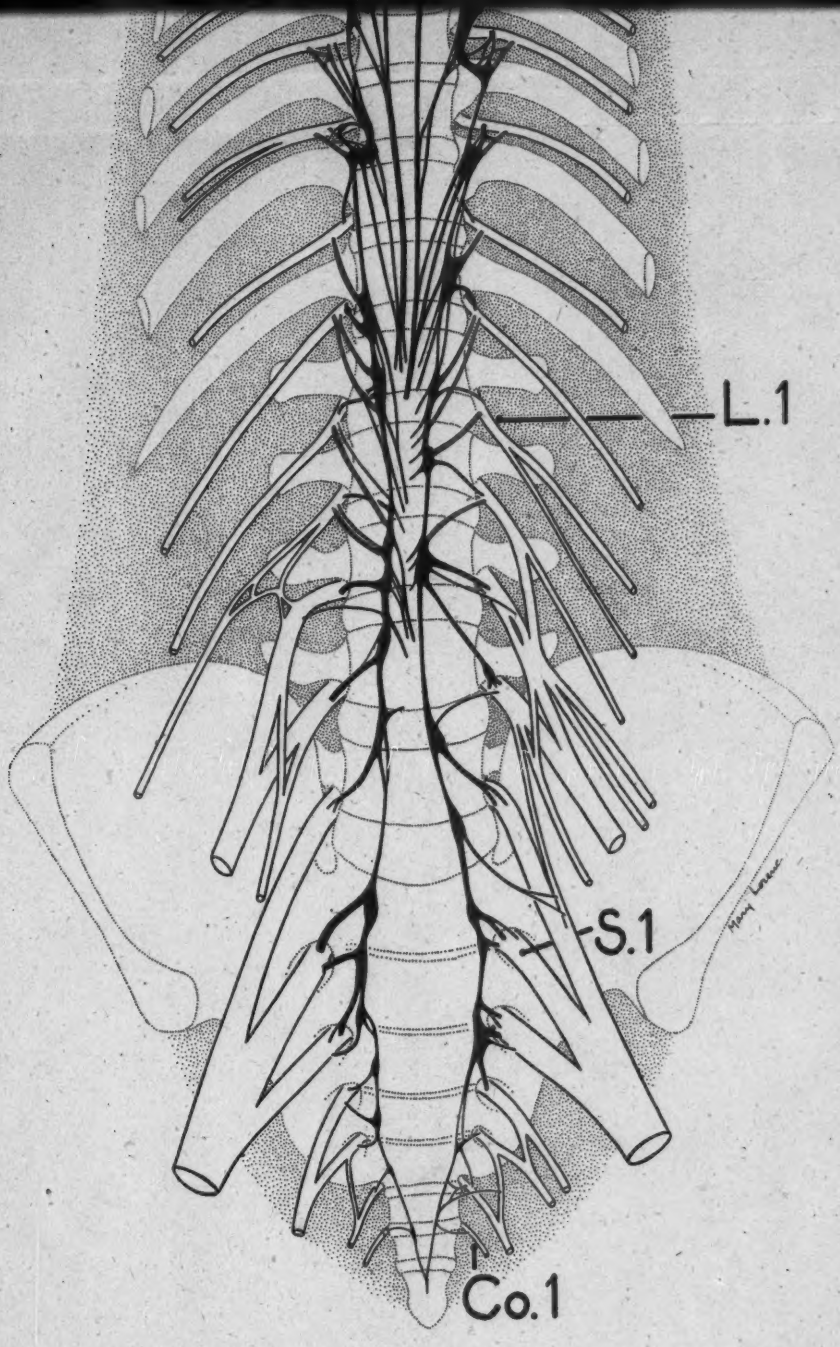


PICK AND SHEEHAN—SYMPATHETIC RAMI IN MAN

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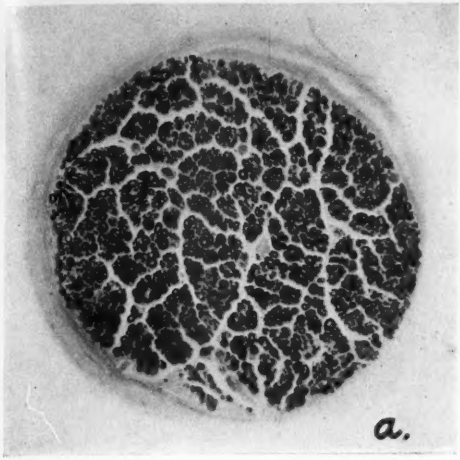


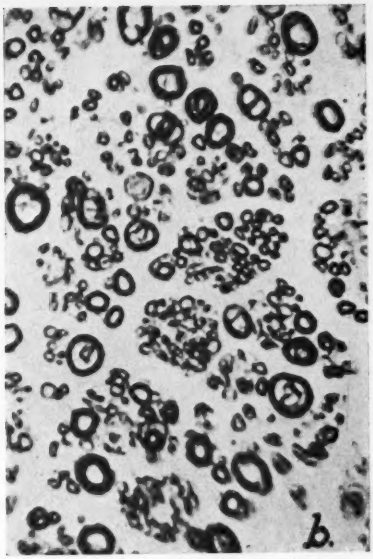


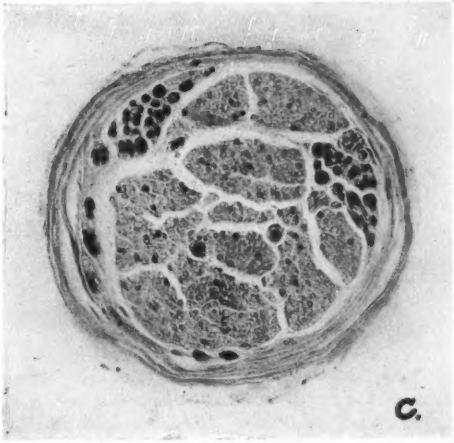


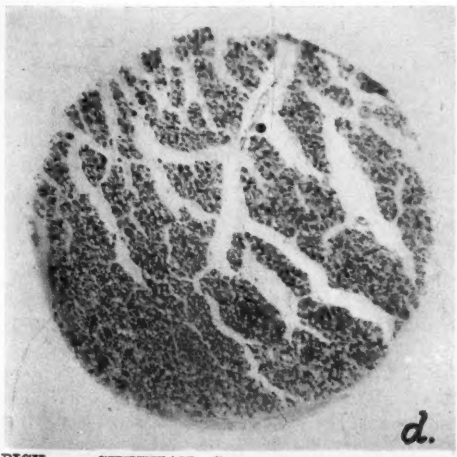


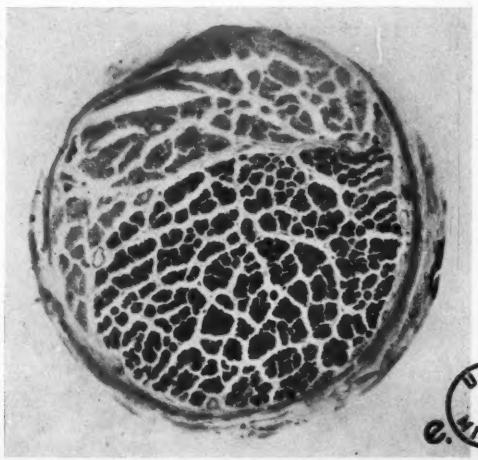


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# THE LYMPHATICS OF THE SYNOVIAL MEMBRANE

By D. V. DAVIES, *Anatomy School, Cambridge*

Although the existence of lymphatic vessels and plexuses in the synovial membrane has been recognized for a considerable time, these structures are still inadequately described and figured. In many cases investigations have not been controlled by histological sections to verify the nature of the injected vessels.

The earliest work on the synovial lymphatics was that of Heuter (1886). He and most other investigators of the last century (such as von Mosengeil, 1876; Hagen-Torn, 1882; Tillmanns, 1876) were concerned primarily with the nature of the synovial cavity in particular relationship to the lymphatic system, and with the much-discussed problem of possible direct communications (stomata) from the joint cavity into the lymphatic vessels. Evidence for the existence of such stomata has been based mainly on silver preparations and is in general inconclusive. The present consensus of opinion is probably against the existence of such stomata, although the ease with which colloidal matter enters the lymph stream from the joint cavity is a feature upon which all workers agree.

Subsequent investigators were concerned chiefly with the pathways and lymph nodes draining particular joints rather than with the configuration of the lymphatic vessels in and around the synovial membrane. Amongst these investigators are Baum (1911, 1927), Mouchet (1911 *a*, *b*), Oschkaderow (1929) and Schdanow (1930).

Chief amongst observations of the synovial lymph vessels and their arrangements are those of Tillmanns (1876) and Magnus (1923). There appears to be some disagreement as to whether the terminal vessels and plexuses can be demonstrated following injection of various colloidal particles and injection masses directly into the joint cavity with subsequent movement of the joint for periods up to half an hour. Tillmanns failed to achieve any success by this method. Baum (1911) was of the opinion that Tillmanns had failed to observe or had wrongly interpreted the vessels which he himself believed must have been injected by this method. More recently Kuhns (1933) wrongly quoted Tillmanns as having succeeded by this method and believed it to be adequate for the demonstration of synovial lymphatics. There seems little doubt that colloidal particles can reach the regional lymph glands following injection of the joint cavity immediately post-mortem, but, as will be seen later, there is a good reason why injection should fail to be an adequate method for the demonstration of the synovial vessels and their plexuses. Furthermore, substances injected directly into the joint cavity become deposited on the surface of the synovial membrane, particularly among its folds, and render subsequent interpretation difficult.

## MATERIAL AND METHODS

Injections were made into the metatarsophalangeal and metacarpophalangeal joints of cattle within 1 hr. of slaughtering. In the earlier experiments the injection was made directly into the joint cavity, in some cases to the point of its distension. The joint was then alternately flexed and extended slowly over a period of ½–1 hr. It was afterwards opened and the synovial membrane examined for lymphatics. In later experiments the joint was opened first and stab injections were made under a low head of pressure directly into various regions of the synovial membrane. The specimen was immersed whole in 10 % formalin for 24 hr. and then examined directly for lymphatic vessels. Portions of synovial membrane and periosteum were removed, and after a further period of fixation in formalin were cleared and again examined with a binocular microscope and in section after light staining with haematoxylin and eosin.

Most of the injections were made with a 50 % dilution of Winsor and Newton's Mandarin Black ink in tap water, using a record syringe and a 20-gauge needle. Other injection masses such as Prussian blue and Berlin blue were also tried but as these seemed to possess no advantage over Mandarin Black they were discarded. In a few cases the lymphatics were successfully injected (after previous delineation of the vascular tree) with carmine gelatine.

## RESULTS

Injections directly into the joint cavity followed by movement failed to delineate the lymph vessels in the synovial membrane, although the injected material was generally found in the larger collecting lymphatic trunks. The explanation of this became obvious when stab injections were made into the synovial membrane. In the latter case movements of the part led to a rapid emptying of the smaller synovial lymphatics. It is known that movement is an important factor in promoting drainage from the joint cavity in the living animal (Adkins & Davies, 1940), as, possibly, is pressure also.

Stab injections into the synovial membrane are

successful in delineating its lymphatic channels in most cases. Over most parts of the synovial membrane the large lymphatics are arranged in the form of a wide-meshed, freely anastomosing, polygonal pattern within which are numerous, blindly ending, smaller tributaries, frequently showing terminal lacuniform enlargements (Pl. 1, figs. 1, 2). The meshes of the lymphatic plexus measure from 2 to 3 mm. across. This plexus in the synovial membrane corresponds to the superficial lymphatic plexus of Tillmanns. Over the more fibrous areas of the synovial membrane, and towards the cartilaginous articular margins, the plexus becomes attenuated; its vessels become finer and fewer, and their anastomoses become less frequent. Unlike the blood capillaries, these fine lymph vessels terminate blindly at some distance from the articular margin.

From the network of lymphatics in the synovial membrane the collecting trunks pass in groups of two, three, or more, along the main blood vessels, generally towards the flexor aspect of the limb. They pass deeply on to the surface of the periosteum and communicate freely with the lymphatic vessels thereof, both where it underlies the synovial membrane and on the shaft of the metatarsal or metacarpal bone (Pl. 1, figs. 3, 4). These lymph vessels on the periosteum are almost invariably injected, and they constitute in the region of the joint the deep lymphatic plexus of Tillmanns.

Where the articular synovial membrane passes over tendons or ligaments in the ox, its lymphatics communicate with those in the areolar tissue which separates and surrounds the tendon or fibrous tissue-bundles (Pl. 2, fig. 5).

Over the intra-articular pads of fat, the lymphatic plexus is poorly marked. Here the vessels are few in number and very wide in calibre; they drain with few anastomoses almost directly into the lymphatics on the periosteum (Pl. 2, fig. 6). Baum's statement that the lymph vessels are particularly numerous over the fat pads cannot be substantiated.

No lymphatic vessels are visible in the villi of the synovial membrane, nor in the cord-like bridges which are sometimes seen at the reflexions of the membrane. This agrees with Tillmanns's observations. It would appear that any activities which the villi might have in the drainage of the joint cavity are achieved through their rich blood capillary bed or their free phagocytic cells.

Passive movement of the joint following injection of the lymphatics is found to empty the synovial plexus quickly and completely. Its effects on the larger collecting trunks, particularly those on the periosteum, are not so marked. This explains the failure to outline the synovial lymphatics by the method of direct injection into the joint cavity followed by movement. Hence it is advisable to fix the joint in formalin for 24 hr. after injection without further manipulation, for thereby the fine lymphatic vessels of the synovial membrane remain injected.

The synovial lymphatic plexus is generally easier to display in the younger animal. In adult joints the collecting trunks are generally injected, but frequently little or none of the synovial plexus shows. The vessels injected in adult joints are generally fewer and finer than in the young animal, in conformity with the general atrophy which overtakes the whole lymphatic system with age.

In histological section the anastomosing lymph vessels of the synovial membrane lie close to the larger blood vessels and are numerous and large (Pl. 2, fig. 7). The smaller blindly ending tributaries generally take a course parallel with the synovial surface, a few, however, passing inwards towards the cavity of the joint (Pl. 2, fig. 7). The synovial lymph channels are neither as numerous nor as superficial as the blood capillaries. They lie in a plane superficial to the elastic lamina which the author has described in the synovial membrane (Davies, 1945), the collecting trunks passing deeply through this lamina on to the surface of the periosteum. The rapidity with which colloidal particles introduced into the joint cavity arrive in the lymphatic trunks and nodes is still a matter for surprise. Very few valves are seen in the synovial lymphatics: they are much more frequent in the accompanying venous channels and in the larger lymphatic trunks on the periosteum with which the synovial lymphatics communicate.

In several cases the injected material was found in the blood vessels of the synovial membrane, with or without an injection of the corresponding lymphatics. These cases were generally identified at the time of injection by the richness and pattern of the vascular net. In some, the circulus articuli vasculosus was completely injected, whilst in others the injection had passed along the veins (perforating veins) in the vascular canal traversing the lower end of the metacarpal bone and thus into the venous sinuses in the bone marrow (Pl. 2, fig. 8). Baum suggested that some of the synovial lymphatics may drain directly into the regional veins. The author could see nothing in the histological sections that was even suggestive of this, although some were cut and mounted in series. It is, however, a possibility that is difficult to confirm or refute. In view of the vascularity of the synovial membrane it is more probable that these injections were made directly into the blood vessels. Surprisingly they did not occur more frequently.

## SUMMARY

The lymphatic vessels of the synovial membrane were studied in the metacarpophalangeal and metatarsophalangeal joints of recently slaughtered cattle,

Fig. 2

Fig. 4

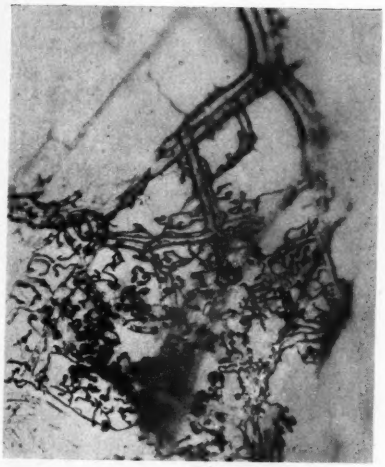

Fig. 1

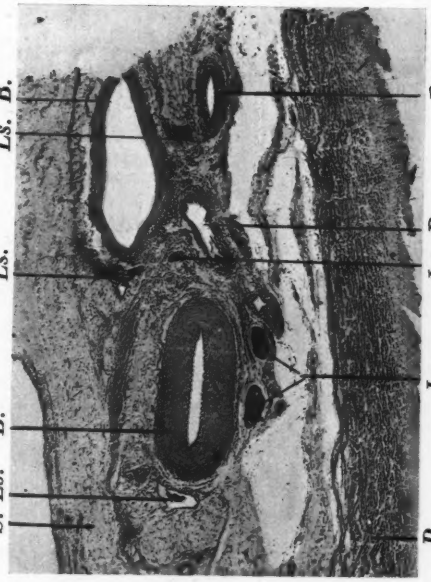


Fig. 3

Fig. 6

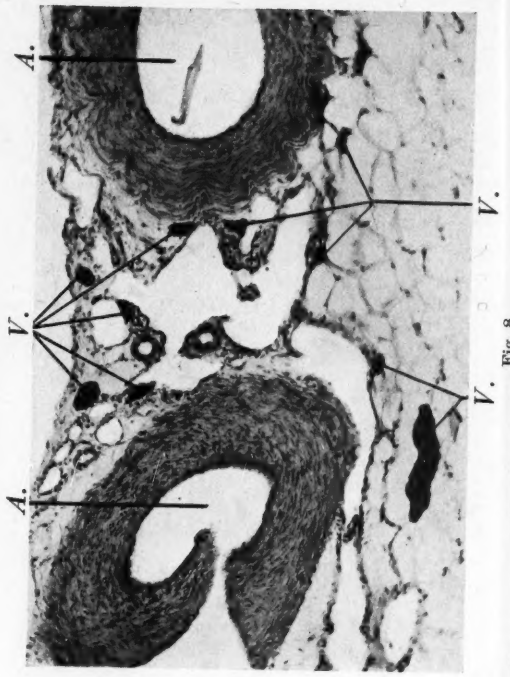


Fig. 8

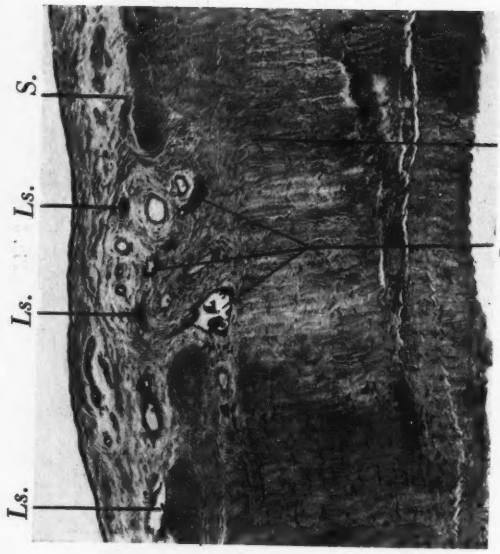


Fig. 5

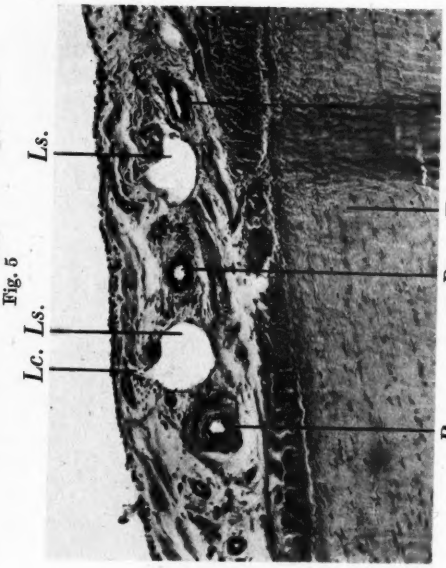


Fig. 7

DAVIES—THE LYMPHATICS OF THE SYNOVIAL MEMBRANE

using 50 % Mandarin Black ink as a medium. To demonstrate the synovial lymphatics, injections directly into the joint cavity followed by movement proved unsuccessful: an explanation is suggested for this. The stab technique proved successful. The synovial lymphatic plexus is described, together with its extra-articular connexions. In some experiments the blood vessels were injected, but no evidence was seen of drainage of the synovial lymphatics directly into the neighbouring veins. All injections were confirmed histologically.

I am indebted to Messrs C. Vangucci, J. F. Simpkin and E. A. King for technical assistance. The photographs are the work of Mr J. A. F. Fozzard.

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## EXPLANATION OF PLATES

### Plate 1

Fig. 1. Lymphatic plexus in the synovial membrane of the metatarsophalangeal joint of an ox. Injected with Mandarin Black ink. ×4·7.

Fig. 2. High-power view of an area from the previous specimen. ×21.

Fig. 3. Section of the synovial membrane and underlying periosteum from the metatarsophalangeal joint of an ox. The large lymphatic vessels on the surface of the periosteum have been injected with Mandarin Black ink from the synovial lymphatic plexus. Stained with haematoxylin and eosin. ×22·5.

Fig. 4. Section of the periosteum from the posterior surface of the metacarpal bone of an ox, some distance proximal to the metacarpophalangeal joint. The lymph vessels on the periosteum contain Mandarin Black ink from the synovial lymphatic plexus. Stained with haematoxylin and eosin. ×84.

### Plate 2

Fig. 5. Section of the synovial membrane and underlying deep flexor tendon from the metatarsophalangeal joint of an ox, showing the lymphatic vessels of the synovial membrane passing deeply to communicate with those in the tendon. The lymph vessels have been injected with Mandarin Black ink. Stained with haematoxylin and eosin. ×84.

Fig. 6. Lymphatic vessels in the synovial membrane overlying a fat pad in the metacarpophalangeal joint of an ox, for comparison with fig. 2. The lymphatic vessels are injected with Mandarin Black ink and are shown at the same magnification as in fig. 2. ×21.

Fig. 7. Section of the synovial membrane from the metacarpophalangeal joint of an ox, showing two large lymphatic channels injected with Mandarin Black ink. A small blindly ending tributary from the more superficial part of the synovial membrane is seen entering one of these large vessels. Stained with haematoxylin and eosin. ×84.

Fig. 8. Section of the bone marrow from the metacarpal of an ox, showing some of the venous sinuses injected with Mandarin Black ink which was injected into the synovial membrane of the corresponding metacarpophalangeal joint. Stained with haematoxylin and eosin. ×84.

Key to lettering. *A.* arteries; *B.* blood vessels; *L.* lymphatic; *Lc.* lymphatic tributary; *Ls.* synovial lymphatics; *Lt.* tendon lymphatics; *P.* periosteum; *S.* synovial membrane; *T.* tendon; *V.* venous sinuses.

# AN EXPERIMENTAL STUDY OF THE REGENERATION OF MAMMALIAN STRIPED MUSCLE

By W. E. LE GROS CLARK, *Department of Anatomy, University of Oxford*

In a previous paper from this department (Le Gros Clark & Blomfield, 1945), which dealt primarily with the efficiency of intramuscular anastomoses, attention was drawn to the fact that ischaemic necrosis in the muscles of rabbits may, under favourable conditions, be followed by extensive regeneration. Indeed, the greater part of the tibialis anterior muscle may become reconstituted in this way. These observations have now been amplified and extended by further experiments, and it is with the results of the latter that the present communication is concerned.

Much study has been devoted in the past to the regenerative capacity of striated muscle, particularly during the latter half of the last century. This work was reviewed at considerable length in 1913 by Küttner & Landois in their monograph on the surgery of striated muscle. The most important of these early observations were those of Waldeyer (1865), Weber (1867), Aufrecht (1868), Neumann (1868) and Volkmann (1893). More recently, similar studies have been made by Forbus (1926), Millar (1934), Kazancev (1935), Pfühl (1937), Mauer (1939) and Levander (1941). Special mention should also be made of the experimental studies *in vivo* of Speidel (1937). All these authors concerned themselves mainly with the initial stages of regeneration, and in general they dealt with material in which the regenerative process was abortive. The massive and practically complete regeneration which was found to occur in the course of some of our own experiments not only permits a more detailed study of the factors which facilitate and direct the regenerating muscle fibres, but has also made it possible to follow the process of regeneration as far as the formation of histologically mature tissue.

The following account of the experiments recently carried out in this laboratory includes a record of the observations made on regeneration in pieces of muscle grafted *in situ* and in crushed muscle, on the degree and extent of regeneration which is found to have occurred several months after ischaemic necrosis, and on the histogenesis of muscle regeneration.

## REGENERATION IN MUSCLE TISSUE GRAFTED *IN SITU*

In the course of the previous work in this department on the vascularization of muscle it was found that ischaemic necrosis in the rabbit's tibialis anterior is rapidly followed by the regeneration of new muscular tissue. However, since a variable amount of the original muscular tissue survived alongside anastomotic channels in the ischaemic zone, the interpretation of the histological material was in some cases doubtful. In order to ensure the complete interruption of the blood supply to a piece of muscle, it was decided to excise a portion completely and immediately suture it in position again. For this purpose, the gracilis muscle was exposed in six rabbits, and a rectangular portion about 15 mm. square was resected, replaced in its original site, and kept in place with sutures. The animals were killed at intervals after the operation ranging from 3 days to 3 weeks. In each case, the animal was injected with bromo-phenol blue 10 min. before being killed in order to assess the vascularity of the graft. After 3 days the graft gave the appearance of a yellow slough, and was entirely unstained in contrast with the blue coloration of the surrounding muscle. Histological sections showed the grafted fragment to be composed almost entirely of dead muscle fibres devoid of nuclei and undergoing fragmentation (Pl. 1, fig. 1). The endomysial cellular elements had also undergone dissolution except for an occasional fibrocyte. At the immediate surface of the graft, however, and also close to the cut margins, a few fibres, or portions of fibres, still survived where their vitality had presumably been maintained by their close relationship to capillary vessels in the surrounding tissues. These surviving fibres in many cases show a granular basophil deposit in the contractile substance, and the endomysial elements investing them have undergone proliferation. Sections stained with Scharlach red also show in some a fine sprinkling of fat granules.

At the end of a week, the grafted fragment has much the same appearance, but now there are signs of regeneration. Fine plasmodial outgrowths are seen extending from the cut ends of the fibres in the muscle surrounding the graft, and growing towards the latter. Inside the graft, also, regeneration is commencing from some of the fibres at the surface which have escaped complete necrotic destruction, and here sarcoplasmic buds are seen sprouting from their remains.

After 11 days, the grafted fragment was found to stain quite distinctly with bromo-phenol blue. Histologically, it shows a considerable amount of fibroblastic tissue, but this is filled with young regenerating fibres disposed in parallel formation

throughout most of its extent. The young fibres are about half the diameter of the normal fibres of the gracilis muscle, and in some of them a faint cross-striation can be detected. The graft is separated from the surrounding muscle by rather dense fibroblastic tissue containing regenerated muscle fibres in tortuous arrangement. It is therefore difficult to determine how much of the regenerated tissue in the graft has arisen from surviving surface and marginal fibres of the graft itself, and how much by the ingrowth through the fibroblastic tissue of sarcoplasmic sprouts from the cut ends of the fibres in the muscle surrounding the graft. The histological appearance indicates that the latter is certainly the source of some of the regenerated fibres, but a large proportion have undoubtedly arisen within the graft itself. At one margin of the graft, short segments of surviving fibres are seen, about 1·5 mm. in length, from both ends of which young fibres stream out in parallel formation. From one end they extend towards the fibroblastic tissue at the margin of the graft, and from the other towards the centre of the graft (Pl. 1, fig. 6).

After 18 days, the graft is found to be filled with young fibres arranged in neat parallel formation with well-developed cross-striations (Pl. 1, fig. 3). There is now remarkably little fibroblastic tissue except at the margin of the graft. Some of the young fibres can be traced in continuity across the junctional zone from the surrounding muscle into the graft and are clearly derived by ingrowth from the former into the latter.

In two experiments, the resected portion of the gracilis muscle was sutured in position at right angles to its original orientation in order to see what effect this might have on the direction of the regenerating fibres. The rabbits were killed 3 weeks after the operation. In both cases considerable regeneration in the graft was found to have occurred, with the new fibres aligned in the direction of the original degenerated fibres. Histological study shows that the new fibres are well developed, with a conspicuous cross-striation, but they have not yet reached their normal diameter. In both cases it is possible to see in some parts of the sections new fibres streaming into the graft across the marginal fibroblastic zone from the stumps of fibres in the surrounding gracilis muscle. Where they enter the edge of the graft, they become deflected at a right angle so as to follow the direction of the original fibres (Pl. 1, fig. 2). It thus appears that the direction of the regenerating fibres from the surrounding muscle into the graft is determined by the structure of the degenerated tissue in the graft and not by their initial direction of outgrowth. It may also be inferred that it does not depend on the direction of tensional forces exerted by the active muscle in which the graft has been sutured, for these are longitudinally disposed.

It is interesting to note that, in the regenerated tissue in the substance of the gracilis grafts, the new fibres not only show a remarkably regular arrangement, but in general they differentiate rapidly and all develop together at an approximately even rate. On the other hand, in the fibroblastic tissue which unites the edges of the graft to the surrounding muscle, the regenerated fibres are seen at all stages of development even at the end of 3 weeks, and here they are also often irregularly shaped and tortuously disposed. The histological picture gives the impression that as soon as the young fibres have penetrated the fibroblastic zone, and extend into the grafted tissue, they grow evenly and with great rapidity, and undergo rapid maturation.

## THE REGENERATION OF MUSCLE FOLLOWING A SIMPLE CRUSH

If a muscle is transected, partly or completely, the cut ends undergo considerable retraction so that reconstitution of the muscle by regeneration is greatly hindered. Several attempts were made (using the sterno-mastoid muscle of the rat) to maintain the cut ends of the muscle in approximation by fixing them in apposition with a plasma clot. However, none of these experiments were successful since the tension on the clot was too great. Accordingly a series of fourteen experiments was completed in which, using the gracilis muscle of the rat, the muscular tissue was interrupted by a simple crush so that the crushed ends of the muscle were kept in position by the connective tissue stroma which still remained uninterrupted. The possibility was anticipated, also, that the collagenous tissue of the endomysium might retain its original pattern in the region of the crush and so provide a supporting framework for directing the subsequent growth of regenerating fibres. In each experiment, the gracilis was exposed and lifted up from the underlying muscles. A transverse linear crush was effected by means of artery forceps in which the teeth had been filed down so as to produce a flat blade 2 mm. in width. The muscle was firmly crushed three times in rapid succession in the same place. Macroscopically this procedure appeared to interrupt all the contractile tissue, leaving a fine translucent sheet of connective tissue binding together the interrupted muscle fasciculi (Pl. 2, fig. 10). The animals were killed at intervals after the operation ranging from 2 days to 3 months.

The immediate effect of crushing was studied histologically in two rats killed within an hour of the operation. At the site of the injury it was found that all the muscle fibres had been interrupted except for occasional damaged fibres which were found still in position crossing the line of the crush. The interrupted fibres had undergone a slight

degree of retraction, but otherwise maintained their alignment. The site of the crush showed extravasations of blood extending for a little distance between the muscle fibres on either side. The collagenous elements were found to be considerably more disorientated than had been hoped. Thus, while the epimysium remained intact, the fine perimysial septa in the muscles were mostly quite disorganized, though a few maintained their alignment in continuity with the undamaged parts of the muscle. In contrast with the fragility of the perimysial tissue, small intramuscular nerves were found still passing without interruption across the line of the crush. The delicate endomysial sheaths of individual fibres and the sarcolemmal tubes nowhere survived except where the remains of crushed muscle fibres still persisted. At the site of the crush, therefore, the following structures persist which might serve to direct the growth of the regenerating fibres from either side: the epimysium, occasional perimysial septa, and occasional damaged but not completely interrupted muscle fibres.

In a gracilis muscle removed 2 days after crushing, the muscle fibres were found to be all completely interrupted at the site of injury. The latter is filled with a fibrinous exudate containing many leucocytes and histiocytes, as well as isolated fragments of contractile substance and newly formed capillary vessels. The cellular infiltration extends for several millimetres among the muscle fibres on either side of the crush. The damaged parts of the muscle fibres have been largely removed, leaving short lengths of sarcolemmal tubes filled with closely packed masses of histiocytes (Pl. 2, fig. 7). No sign of active regeneration can be observed.

At the end of 3 days the histological picture begins to change rapidly. The fibrinous exudate at the site of the crush is now invaded by fibroblasts in addition to leucocytes and histiocytes. For the most part the fibroblasts show no definite orientation, but occasionally they are disposed in long strands, apparently in relation to the remains of perimysial septa. From the stumps of the muscle fibres on either side of the injury, plasmodial outgrowths of sarcoplasm are seen extending along the sarcolemmal tubes from which damaged segments of the fibres have been removed. Thus the general appearance is given of numerous incipient buds of young muscle fibres sprouting out in parallel formation towards the centre of the crushed zone from either side.

After 4 days the fibroblastic tissue has considerably increased in the crushed zone, but the latter also contains numerous fine strands of newly regenerated muscle fibres which have now met and overlapped each other in their growth from either side. The young fibres tend to run in closely packed bundles, but the latter do not remain separate throughout their extent for they often anastomose with each other in the centre of the crushed area (Pl. 1, fig. 4).

In the 7-day experiment the sections show an excessive amount of fibroblastic tissue deposited at the site of the injury to the muscle, which has evidently prevented the regeneration of new muscle fibres across this region. In the 10-day experiment, numerous young fibres are seen traversing the crushed zone, interweaving in a complex manner in the fibroblastic tissue. As in the previous case, this tissue seems more dense than in the other experiments of the series, and this provides a less favourable opportunity for muscular regeneration.

In the later experiments (from 3 weeks up to 3 months after operation) the fibroblastic tissue at the site of the crush appears to be much reduced in comparison with the shorter-term experiments, either because of its removal and replacement by regenerated muscle tissue, or perhaps because there had been less haemorrhage at the site of the injury. In all these cases, the two ends of the muscle have become firmly united across the crushed zone by muscular tissue. The latter, however, is not laid down in regular arrangement. In one experiment (in which the muscle was removed 6 weeks after crushing) the individual fasciculi were found to preserve their longitudinal pattern across the line of injury, presumably because in this case the perimysial tubes of connective tissue were preserved in continuity more completely than usual (Pl. 2, fig. 11). But within each fasciculus, the individual muscle fibres are in all cases considerably disorientated. The regenerated fibres from either side inosculate and interweave with each other so that they become knitted together in a most intricate manner. It is not uncommon also to find fibres bifurcating, in some cases more than once. Direct anastomoses may also occur between adjacent fibres, but this appears to be extremely unusual (Pl. 2, fig. 13). In the gracilis muscle removed 3 months after crushing, superficial inspection revealed little signs of the injury. Histological sections show that complete muscular union has been restored across the line of crush, but here the muscle fibres are for the most part disposed in inextricable confusion (Pl. 2, fig. 9). In some regions, however, they extend in more or less parallel formation from one side to the other, and it is presumed that this alignment is related to the preservation of collagenous septa in the crushed zones.

Even at the end of 3 months, the regenerated fibres are found still to be in all stages of development and of widely different sizes, and they also show various depths of staining. While many are normal in diameter, staining properties and the disposition of their nuclei, others are extremely fine, less than 5 $\mu$ in diameter, with central nuclei

arranged in characteristic rows (Pl. 1, fig. 5 and Pl. 2, fig. 12). These fibres are often seen clinging to the surface of fibres of normal size, and they can sometimes be traced as outgrowths from normal fibres in the vicinity of the crushed zone. They are also sometimes seen in closely packed bundles, suggesting a process of fasciculation such as occurs in regenerating nerve fibres. In some of the fibres of normal size the nuclei are still very numerous, and are arranged in close linear formation at the surface beneath the sarcolemma. Except for the finest fibres, cross-striation is well developed in all the regenerated muscle tissue. No certain evidence was found in any of the experiments that newly formed fibres growing out from either side unite in continuity as single uninterrupted fibres. Where, exceptionally, this appears to be the case, it is not possible to exclude the chance that some of the original fibres had escaped complete interruption when the muscle was crushed.

## THE RECONSTITUTION OF THE TIBIALIS ANTERIOR, FOLLOWING ISCHAEMIC NECROSIS

Attention was drawn in the previous report from this department to the rapid regeneration of muscular tissue which occurs in the rabbit if the lower two-thirds of the tibialis anterior muscle are rendered necrotic by devascularization. It was found that by the end of 3 weeks the necrotic zone becomes almost completely filled with young fibres which have streamed down as fine protoplasmic strands from surviving fibres in the upper third of the muscle. However, the ultimate fate of this new muscular tissue was not determined. There was also some element of doubt as to the exact degree of devascularization and necrosis in the experiments recorded, since this was found to show some variation in different animals after ligature of the main vessels of supply to the tibialis anterior. Accordingly, more experiments of a similar type were carried out in which the degree of devascularization was determined by the injection of bromo-phenol blue and regeneration was allowed to continue for a longer time.

In two rabbits the main vessels to the tibialis anterior were ligatured on both sides. Two days later, the muscles were exposed and bromo-phenol blue injected into the ear vein. In both cases approximately the upper third of each muscle was stained a normal blue colour while the lower two-thirds remained unstained, with a sharp line of demarcation between the two parts. This contrast was recorded photographically (Pl. 4, fig. 24). Since previous work in this department showed that, in a devascularized muscle which remains unstained after intravenous injection of bromo-phenol blue 2 days following devascularization, the muscular tissue is almost entirely necrotic, it can be assumed that similar conditions had been induced in the present cases. Three weeks later the tibialis anterior of one side was removed from each animal. One animal was killed 3 months and the other 4 months after the initial operation, and the tibialis anterior of the other side removed for histological study.

The histological appearance of the tibialis anterior removed 3 weeks after devascularization was found to be approximately the same in both animals. Throughout rather less than the lower two-thirds of the muscle, the necrotic tissue had been almost entirely removed and its place taken by young regenerated fibres. The latter are of fine calibre, regularly arranged in parallel formation, and show well-developed cross-striations. Their appearance is quite similar to those shown in Pl. 3, fig. 18. A transverse section of a tibialis anterior muscle at a similar stage of regeneration is shown in Pl. 4, fig. 20, where the increased amount of endomysial tissue is also well demonstrated. Occasional old fibres which have survived are to be seen near vessels in the central axis of the muscle or close to the epimysium on the surface, but these are inconspicuous and difficult to find in the sections. Most of the new fibres are in approximately the same stage of differentiation except in patches of fibroblastic tissue near the surface of the muscle where the development of a few isolated fibres has evidently been delayed.

The tibialis anterior muscle removed 3 months after operation showed macroscopically some degree of shrinkage. Histologically the lower two-thirds of the muscle shows some local patches of fibrosis and fatty infiltration, but apart from these it is made up of muscular tissue of approximately normal appearance. The tibialis anterior removed from the other animal 4 months after devascularization appeared normal to superficial inspection. Histologically it shows complete reconstitution of the lower two-thirds in so far as this is almost entirely made up of muscular tissue of healthy and mature appearance. Fatty infiltration is absent except only for a minute amount between some of the muscle fasciculi. The fibres are in general of normal size, though somewhat more variable in diameter than those of a normal tibialis anterior. They are also more richly nuclear, and at the lower end of the muscle are not quite so regularly parallel in their formation, showing a tendency to interweave. A careful search through the sections occasionally brings to view a bifurcating fibre, but such an appearance is remarkably rare. At the surface of the muscle, also, and near the lower end, it is possible to find a few fibres of embryonic type. The endomysial tissue is slightly more abundant in the regenerated tissue (as demonstrated by staining with Van Gieson) than in the normal

muscle, but it is not nearly so abundant as in the regenerating muscle 3 weeks after devascularization. Thus it appears that some of the connective tissue which is formed in the early stage of regeneration may later be removed. Lastly, the vascularity of the whole muscle appears somewhat greater than normal. Apart from these unusual characters (which are by no means obtrusive) the lower two-thirds of the tibialis anterior in this 4-months' experiment has a normal histological structure. Thus it is possible to state that, in the rabbit, under favourable conditions the greater part of the tibialis anterior muscle can undergo practically complete reconstitution after ischaemic necrosis by the regeneration of new muscle fibres.

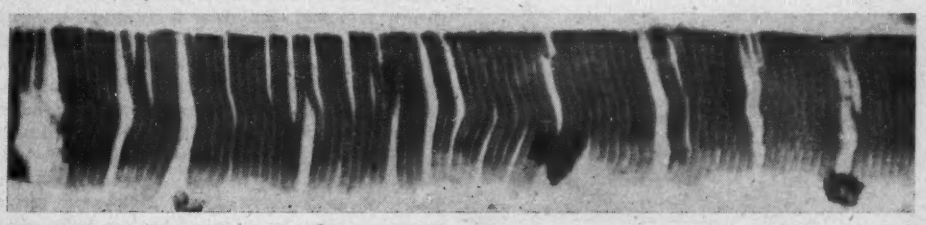
## THE HISTOGENESIS OF MUSCLE REGENERATION

In all our experiments involving mechanical or ischaemic destruction of muscle, no regeneration was observed until the third day. The histological changes which occur during the first 2 days are those of degeneration and reaction, that is to say the hyaline degeneration and fragmentation of injured muscle fibres, an endomysial oedema, and the invasion of the damaged area by large numbers of cells which are mostly polymorphonuclear leucocytes but also include histiocytes. In the case of fibres which have been crushed, the sarcolemmal tubes of the damaged segments of the muscle fibres become rapidly filled with histiocytes, and by the second day they are tightly packed with these cells. In this manner the retraction clots which are formed immediately on crushing are removed. These 'histiocyte tubes' are seen extending into the zone of crushing from the stumps of muscle fibres on either side (Pl. 2, fig. 7). The earliest stages of regeneration of the injured muscle fibres are best seen in the crushing experiments, for here the cellular reaction following the injury is considerably less than that which follows cutting or ischaemic necrosis. Even in the crushed tissue, however, the fibroblastic activity, the capillary proliferation and the infiltration by leucocytes and histiocytes give a confused histological picture which is not easy to analyse.

The first sign of regeneration is a plasmodial outgrowth of granular sarcoplasm which protrudes from the stump of the uninjured part of the fibre. Where the sarcolemmal sheath has preserved its integrity, the outgrowth extends along it in an even course forming a cone-shaped mass with a blunt tip (Text-fig. 1 B and Pl. 2, fig. 8). The nuclei within the outgrowth (which may be numerous) tend to be arranged in parallel rows, and the sarcoplasm often shows a longitudinal fibrillation. Where the sarcolemmal sheath is distorted or damaged, the outgrowth is correspondingly irregular. Frequently the growing end is scalloped where the sarcoplasm comes up against histiocytes or fragments of injured muscle fibre (Text-fig. 1 A). As it extends farther, the plasmodial outgrowth, while confined by the still intact portion of the sarcolemmal sheath, sometimes shows a vacuolated appearance where it 'flows round' and engulfs cellular debris and fragments of dead muscle fibres (Text-fig. 1 C). However, there is no reason to suppose that the plasmodial outgrowths are capable of active phagocytosis or that they play any part in the removal of necrotic material. In areas where haemorrhage has occurred, histiocytes may be seen filled with pigment granules, but the sarcoplasmic buds in the same region have not been observed to show such a reaction. Where the plasmodial outgrowth extends beyond the limits of a clearly defined sarcolemmal sheath, it sends out a slender pointed process which appears to push its way into the surrounding tissues (Text-fig. 1 C, D). In such a process, nuclei are characteristically seen arranged along its length in single rows. More than fifty such nuclei have been counted forming an uninterrupted chain. These embryonic muscle fibres are sometimes to be seen in close contact with the

Text-fig. 1. Early stages of the regeneration of muscle fibres at the margin of the crushed zone of a gracilis muscle. A, B and C were drawn from a section 3 days, and D 4 days after crushing. ×120 approximately.

surface of adjacent normal fibres, and frequently several of them are found clinging together in a closely packed bundle. Their further history depends on the local conditions. Where they enter dense fibroblastic tissue (which is their usual fate in muscle injuries) they become grossly distorted in their course, and in isolated sections short segments, devoid of a sarcolemmal sheath and apparently lying free in the surrounding tissue, may be seen disposed in all directions. Normally, the tip of the outgrowth is extremely fine and pointed, but if it meets with obstruction in its continued growth the terminal part may spread out (in a manner suggesting the damming-up of a stream) to form large multi-nucleated masses. This phenomenon is well illustrated in one specimen of the tibialis anterior in which the newly growing muscle fibres came up against a barrier formed by the tendon on the surface of the muscle, giving rise here to a series of such multi-nucleated masses. In the gracilis grafts (from the 4th day after the operation) giant multi-nucleated cells form a very conspicuous mentation within the first 2 days. It is interesting to note, however, that they do not lose their cross-striations. Indeed, this even becomes accentuated in many of the fibres and is still perfectly distinct in fibres which have been dead for at least 10 days. Moreover, under the action of fixatives, the necrotic fibres show a strong tendency to fracture transversely along planes of cleavage represented by the isotropic discs, so that adjacent anisotropic discs may become separated from each other in the form of conchoidal plates (Text-fig. 2). This phenomenon may perhaps be regarded as a confirmation of the view which maintains that there is a real difference of organization between the anisotropic and the isotropic discs. It seems probable that the effect of the formalin (which was used as a fixative) has been to cross-link those chain molecules which are in more perfect parallel organization (for it is well recognized that formaldehyde builds actual chemical cross-linkages between neighbouring protein chains). In some cases the fibres show a zigzag splitting so as to produce a series of 'steps' at the line of frac-



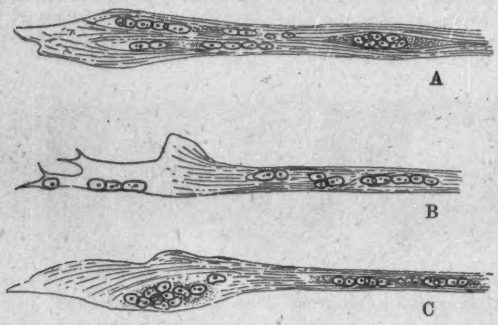
Text-fig. 2. A dead muscle fibre from a gracilis graft 11 days after its insertion. Note the preservation and accentuation of the cross-striation, and the tendency to transverse fracture along cleavage lines represented by the isotropic discs. ×730.

feature in the dense fibroblastic tissue which develops at the margin of the graft and in the neighbourhood of sutures. They appear finally to lose their connexion with the original outgrowths and to form isolated elements. Quite similar formations have been described in previous studies of muscle regeneration and by Bullock & Curtis (1922) and Lewis (1943) in transplants of a rhabdomyosarcoma.

In the massive regeneration of muscle fibres which occurs in the devascularized portions of the tibialis anterior muscle the outgrowth of young muscle fibres is facilitated and directed by a number of factors. In the first place, although there is an immediate fibroblastic reaction at the junction of the ischaemic with the intact part of the muscle, it is by no means so dense as that which occurs at the cut margins of grafts or in areas of muscle destroyed by chemical agents or violent injury. Thus the initial plasmodial outgrowths penetrate to the margin of the necrotic zone quite early. In the necrotic area the devascularized muscle fibres lose their nuclei and begin to undergo fragture. Here it may be supposed that the cross-linkage is imperfect, for the two resolved components of an oblique stress have succeeded in bringing about both longitudinal and lateral rupture (Astbury). Incidentally, the separation of the anisotropic discs argues strongly against the conception that the striations of a muscle fibre are merely the optical aspect of a continuous spiral formation.

When the contractile substance of a fibre thus undergoes a process of fragmentation, its sarcolemmal membrane may remain intact for a week or so over a considerable part of its length, forming what appears to be an empty tube where fragmented segments of the contained fibres have retracted from each other (Pl. 1, fig. 1). While these necrotic changes take place in the muscle fibre, the endomysial cellular elements also undergo dissolution and, although occasional nuclei of fibrocytes are still to be seen in areas which have been necrotic for 10 days, most of them disappear altogether. On the other hand, the muscle fibres become separated from each other by an endomysial

oedema, and in the spaces so formed a slight cellular exudate appears, consisting of a few scattered leucocytes, and an occasional histiocyte. Perimysial septa of collagenous tissue (which are rather inconspicuous in the tibialis anterior muscle of rabbits) persist in the devascularized zone, and thus play a part in maintaining the original connective tissue framework of the necrotic part of the muscle. The immediate reaction at the junctional zone between necrotic and normal muscle is the invasion and phagocytosis of the contractile substance of the dead fibres by clumps of histiocytes and the invasion of the endomysial exudate by large numbers of fibroblasts. The latter, by their rapid proliferation, spread down in the spaces between the muscle fibres on the surface of the old sarcolemmal sheaths some way beyond the advancing line of histiocytic phagocytosis occurring within the sheaths (Pl. 3, figs. 14, 15). In this manner




Text-fig. 3. The expanded growing tips of regenerating muscle fibres, from a tibialis anterior muscle 10 days after devascularization. ×300 approximately.

each dead fibre becomes progressively enclosed in a tube of new endomysial tissue, while the old sarcolemmal sheaths are reinforced or, where deficient, reconstructed. The importance of the new endomysial tubes, thus built up on a scaffolding of dead muscle fibres, lies in the fact that they evidently provide channels which facilitate and guide the newly growing fibres. The latter, which, as already noted, sprout out from the stumps of the surviving portions of the old fibres, rapidly extend towards the necrotic zone. In sections through the tibialis anterior 8 days after devascularization, the histological picture of the replacement of old by new fibres is striking. The new fibres are seen streaming in parallel formation through fibroblastic tissue to the zone of necrotic muscle (Pl. 3, fig. 16). Each young fibre enters one of the newly constructed endomysial sheaths and appears to flow down it until it makes direct contact with the end of the old dead fibre which still remains and which itself is capped by a clump of active histiocytes in the process of removing it (Pl. 3, fig. 17). It is evident that as fast as an old fibre in this manner undergoes its progressive dissolution by histiocytic activity, the advancing tip of a new fibre follows it up. Thus the original pattern of fibre architecture is reproduced by the regenerated tissue. The appearance of the advancing tips of the new fibres is shown in Text-fig. 3 and in Pl. 3, fig. 17. The tip becomes somewhat expanded, and is often scalloped where it moulds itself round the blunt end of the dead fibre and its associated clump of histiocytes. It is composed of clear sarcoplasmic material which shows a longitudinal striation. It may contain clusters of nuclei, but more frequently these are absent towards its actual extremity. Sometimes the expanded tip sends out fine processes which penetrate between the histiocytes covering the adjacent end of the old fibre which is in course of replacement (Text-fig. 3 B).

If for some reason the penetration of the regenerating fibre into the newly constructed endomysial sheath is delayed, the latter is left as an empty tube when the remains of the old fibre are completely removed. This is well shown in the microphotograph, in Pl. 4, fig. 21, of a section taken through the lower end of a tibialis anterior muscle 23 days after devascularization. The tubes are held open at intervals by short segments of necrotic fibres which still remain. Elsewhere, as can be seen in Pl. 4, fig. 22, the tubes are empty and collapsed. The tips of the regenerating fibres which are growing into such empty tubes do not show the terminal expansions already described, but are finely pointed. The terminal expansions may thus be regarded as the result of a temporary obstruction when the regenerating fibre comes up against the stump of an old unabsorbed fibre. The new endomysial tubes, filled by newly grown muscle fibres, are well seen in the microphotographs (Pl. 4, fig. 20) of a transverse section through the lower part of a tibialis anterior muscle 21 days after devascularization.

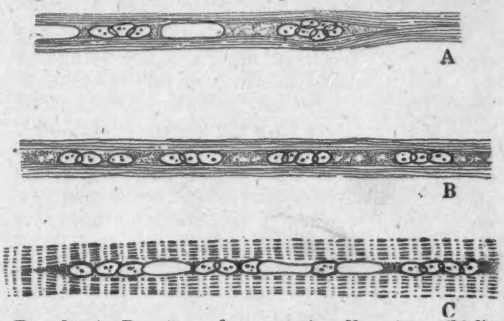
The directive action of endomysial tubes in the regeneration of muscle fibres is further emphasized by the experiments in which grafted pieces of gracilis muscle were turned at right angles to the normal position, with the result that ingrowing fibres from the surrounding muscle deviated from their original course and followed the direction of the reorientated fibres in the graft. Attention may also be drawn to the fact that, when regenerating muscle fibres grow into muscle defects which are filled with a serous exudate or a blood clot, they course in various directions with no predominant orientation. This is shown by the results of the crushed gracilis experiments recorded above, where the site of the injury becomes partly occupied by extravasations of blood or a fibrinous exudate. In these regions, the new fibres interlace in an intricate pattern, frequently branching and occa-

sionally anastomosing with each other. Similar observations have been made by Kazancev (1935). Incidentally, also, it may be noted that the tensional forces exerted by the gracilis muscle pulling longitudinally on the crushed zone are not adequate for determining the orientation of the regenerated muscle fibres in the absence of endomysial tubes constructed on the remains of old fibres. It seems probable that the endomysial tubes also play a part in the rapid and even maturation of new fibres, for in the crushed zones in the gracilis muscles these fibres show a great variation in their size, the degree of their development, and their staining properties, even at the end of 3 months. This picture is in strong contrast with the regenerated tissue in the gracilis grafts and in the devascularized parts of the tibialis anterior, for here the fibres develop rapidly and at a fairly common rate, and also stain evenly.

An attempt was made to study further the directional growth of regenerated muscle fibres in the absence of an endomysial framework by filling defects in the tibialis anterior with rabbit plasma clot. It was expected that the sarcoplasmic outgrowths from the muscle fibres at the margin of the defect would penetrate the clot. The experiment was carried out on two rabbits. In both cases, after 7 and 10 days respectively, the clot was found to contain many fibroblasts and histiocytes, but no regenerating muscle fibres. The latter appear in the sections to be held up in the fibroblastic tissue at the margin of the clot, where they terminate in bulbous multi-nucleated masses of sarcoplasm extending in different directions.

The role of the endomysial tubes, serving as guides for the new muscle fibres, parallels the role of the endoneurial tubes in nerve regeneration, except that in the case of damaged muscle the tubes are constructed anew by fibroblastic activity. As long ago as 1868, Neumann drew attention to this activity and described how it leads to the formation of endomysial sheaths enclosing the young muscle fibres after regeneration. But he did not emphasize the important role which they appear to play in directing the growth of these fibres. More recently Pfühl (1937) has stressed the importance of the sarcolemmal sheaths as functional guides for regenerating fibres. He finds these sheaths to be very resistant to inflammatory processes in small localized injuries, but that when the young muscle fibres (with which they become filled during the process of regeneration) attain to a normal diameter, they undergo dissolution and new sarcolemmal sheaths are constructed. He further considers that complete regeneration occurs only when the connective tissue of the damaged muscle has survived and the guiding sarcolemmal sheaths preserved. However, in the more extensive regeneration reported in the present paper (as noted above) endomysial tubes are formed anew on a scaffolding of the old necrotic fibres by the preliminary invasion of fibroblastic tissue, and the sarcolemmal sheaths of the old fibres are thereby reinforced or reconstructed in advance of the growing new fibres.

As already mentioned, longitudinal striation may be evident in the sarcoplasm of regenerating fibres as soon as they appear in the form of buds sprouting from the stumps of old fibres. At this stage, however, it is faint and shows no very regular arrangement. By the second day of growth, regular longitudinal striae or myofibrillae are very distinct in the peripheral zone of the young fibre, and the latter commonly appears tubular in character with a central core filled with granular sarcoplasm and containing nuclei disposed in a single row, or in a regular series of small clumps. The central core is




Text-fig. 4. Drawings of regenerating fibres in a tibialis anterior muscle 10 days after devascularization. The young fibres have a tubular appearance, with a central core of granular sarcoplasm containing nuclei arranged in regular groups. In A and C the vacuole-like spaces referred to in the text are shown in the central core. In C the myofibrillae have already developed a distinct cross-striation. ×500 approximately.

not necessarily continuous throughout the length of the growing fibre, for in the intervals between the nuclear clumps the myofibrillae in many cases appear to extend throughout the whole thickness. On the other hand, the nuclear clumps often give the appearance of pushing aside the myofibrillae so that the latter become deviated from their straight longitudinal course. In many fibres of 5 or 6 days' growth, the central core of sarcoplasm may be occupied here and there along its course by clear spaces of vacuolar appearance which extend between adjacent clumps of nuclei. Their appearance is shown in Text-fig. 4 A and C, and it will be noted that, where they extend up to the nuclei, the nuclear membrane is indented in a manner which suggests that the spaces contain a fluid under positive pressure. Many of the regenerating fibres have lost their 'tubular' character by the end of the first week and the nuclei tend to

shift from a central to a peripheral position. Even before this stage, however, the myofibrillae may show cross-striations. This character has been found to be well developed as early as the sixth day of growth, and after 2 or 3 weeks it is very distinct (Pl. 1, fig. 3). The growth in thickness of the new fibres appears to proceed slowly during the first few weeks, and does not affect all the fibres quite uniformly. The transverse sections of the lower part of tibialis anterior shown in Pl. 4, fig. 20, illustrates the result of 3 weeks' growth, and it will be noted that there is some variation in the diameters of the new fibres, though in general they have reached the same stage of differentiation. Direct measurements on sections of regenerating muscle show that after 10 days to 3 weeks the average thickness of the new fibres is approximately one-third of the normal. By 3 months, the fibres have increased to their normal thickness.

The rate of longitudinal growth of regenerating muscle fibres has been approximately estimated by taking measurements in favourable sections in which the new fibres can be followed throughout most of their extent from the stumps of old fibres. In estimating the rate of growth, it has been assumed (on the basis of our histological observations) that the active outgrowth of new fibres does not occur during the first 2 days following injury to the muscle. In material from three experiments, the following data were obtained. In one case after 6 days of active regeneration, some of the new fibres were found to have reached a length of at least 9 mm., and in two other cases, after 8 and 9 days of regeneration respectively, a length of 8 mm. Thus it may be inferred that the maximum rate of growth is at least 1–1·5 mm. a day. It is very probable that the maximum rate is actually somewhat greater, since some of the regenerating fibres measured could not be traced in individual sections to their ultimate terminations.

Our extensive histological material of regenerating muscle has convinced us that the new muscle fibres are probably entirely formed as direct and continuous outgrowths derived from old muscle fibres. It remains possible, as often suggested by earlier observers and also tentatively suggested in the previous communication from this laboratory, that myoblasts are also derived from disintegrated and isolated fragments of damaged fibres so that they are not found in direct continuity with old fibres. However, a more detailed study has provided no convincing evidence for this. Individual sections often give the appearance of isolated myoblasts scattered in the fibroblastic tissue at the site of regeneration, but serial sections indicate that the isolation is only apparent, and that the 'myoblastic' elements can be traced ultimately, as long protoplasmic strands, into continuity with old fibres. In some cases microphotographs of successive sections were taken and compared in order to establish this point. Quite recently, Levander (1941, 1945) has put forward the thesis that in the course of the regeneration of mammalian muscle new myoblastic elements arise by the differentiation of generalized connective tissue cells, which he supposes results from a process of induction. In support of his contention, he gives a microphotograph showing apparently isolated myoblasts arranged (as he describes) like a shoal of fish in the granulation tissue. We have frequently seen this appearance in our preparations but serial sections show that probably in all cases it is simply due to the oblique sectioning of long continuous strands which can be ultimately traced without interruption to the stumps of pre-existing fibres. If there has been much distortion of the tissues (as has evidently occurred in Levander's material, in which necrosis was induced by the intramuscular injection of alcohol), the connexion between the young fibres and pre-existing fibres may not be easy to establish without the detailed study of large numbers of serial sections. Levander also supports his interpretation by the appearance of young muscle fibres in the neighbourhood of pieces of muscle transplanted into the subcutaneous tissue. He seems to assume that the original fibres of the transplant undergo complete necrosis. In fact, as already noted in the description of our experiments with gracilis grafts, some of the muscle fibres at the surface of a transplant may survive and give rise to young regenerating fibres. It should also be emphasized that the outgrowth of new muscle fibres proceeds with considerable rapidity, so that a single section may show newly formed fibres at some distance from the position of pre-existing fibres, suggesting an independent origin. Our experiments do not, of course, exclude the possibility of such an origin, but this could only be established by experiments of a more critical character.

From time to time, ever since Weber in 1867 supposed that in certain circumstances muscle cells might become transformed into 'inflammatory cells', the suggestion has been made that scavenging cells may arise from the budding and separation of the sarcoplasmic elements of damaged muscle fibres and that these cells play an important part in the removal of the dead contractile substance. The histological appearance in sections at the site of muscle injury not infrequently suggests such a process. On the other hand, the invasion of the area by masses of histiocytes, which rapidly penetrate into the sarcolemmal sheaths and enter the contractile substance of necrotic fibres, often renders the interpretation of the picture extremely difficult. Thus it may not be easy to decide whether clumps of cells embedded in the stump of a degenerating fibre are actually derived from the sarcoplasmic elements of the fibre or are histiocytes which have

secondarily invaded it. There can be no doubt, however, that in certain circumstances the remains of a damaged muscle fibre may give rise by budding to independent cellular elements, for quite convincing histological pictures of this process have occasionally been observed in our preparations. This process is illustrated in Pl. 4, fig. 23, which shows rounded mononuclear elements budding off from the stump of a damaged (but not completely destroyed) muscle fibre. Some of them are still attached to the stump, forming a pedunculated mass, and their cytoplasm contains an extension of myofibrillae from the parent fibre. When isolated, they appear as rounded cells with a central nucleus and a clear eosinophilic cytoplasm. The fate of these elements cannot be determined from our material, but there is no certain evidence that they form independent myoblastic tissue or that they play any part in the removal of necrotic muscle fibres.

In his study of regenerating muscle, Forbus (1926) found that during the early stages the sarcolemmal tubes of damaged fibres contain, besides phagocyte cells, morphologically different cells which are unquestionably of muscle origin. These cells do not take up vital dyes previously injected, but if the administration of the dye is continued during the experiment they store it in large quantities. Pfühl (1937) has also described the formation of cells resembling histiocytes from the sarcoplasmic elements of muscle fibres during the type of degeneration called by him 'dissociative'. He reports that these cells, which he terms 'muscle corpuscles', survive for a few hours.

The conception that degenerating muscle fibres may give rise to histiocytic elements is difficult to accept in view of the highly specialized nature of striated muscle. Nevertheless, it receives some support from recent work by Chevremont (1940) who claims to have observed the direct transformation *in vitro* of myoblastic cells into typical histiocytes.

As the young regenerating fibres grow down into the necrotic zone of a devascularized muscle it is not uncommon for them to divide so that two or three new fibres are found to be formed from the stumps of a single old fibre. This phenomenon has already been noted and figured by Forbus (1926). Thus the reconstituted tissue in the necrotic zone of a muscle (at least in early stages of regeneration) may contain many more fibres than the normal muscle. In one experiment, in which the tibialis anterior of one side had been devascularized for 21 days, transverse sections through the regenerated lower part of the muscle were compared with equivalent sections of the normal muscle of the other side (see Pl. 4, figs. 19, 20) and counts of muscle fibres were made. It was found that the regenerated fibres on the operated side were approximately 50 % in excess of those of the normal side. On the other hand, in the experiment in which regeneration was allowed to proceed for 4 months, the tibialis anterior was of approximately normal bulk and the sections showed the regenerated portion to contain muscle fibres of approximately normal diameter. It seems probable, therefore, that at least some of the excess fibres which are first formed do not gain maturity. On the other hand, in the 4 months' material, bifurcating fibres are still occasionally to be seen.

A careful study of all the histological material of regenerating muscle indicates that, as many other observers have noted, nuclear proliferation in the growing fibres takes place normally by amitosis. Occasional mitotic figures are seen in the sarcolemmal sheaths occupied by regenerating fibres but it appears probable that in many instances these belong to histiocytes which still remain within the sheaths. In one experiment in which the gracilis muscle of a rat had been crushed 4 days previously, colchicine was administered to the animal 10 hr. before death. As a result the sections showed many mitoses in the connective tissue elements at the site of the injury, but no mitoses could be definitely assigned to the sarcoplasmic outgrowths of regenerating fibres. It may be inferred, therefore, that if mitosis does occur in the regenerating fibres it is an unusual phenomenon.

It is interesting to note that a short segment of a surviving old muscle fibre may show considerable lengths of regenerating fibre sprouting out simultaneously from both ends. As already noted, this phenomenon was observed in a gracilis graft which was examined 11 days after its implantation. At one point near the surface of the graft segments of old fibres about 1·5 mm. in length were found to have maintained their vitality. From each end young fibres had grown for a distance of several millimetres into the necrotic portion of the graft, and, as far as could be judged from this single specimen, the degree of regeneration was approximately equal at both ends (Pl. 1, fig. 6).

## SUMMARY OF CONCLUSIONS

1. Pieces of the gracilis muscle of rabbits resected and grafted *in situ* undergo complete necrosis except for a few fibres at the surface and near the margins.

2. Within 2 weeks regenerating muscle fibres are present in the graft in regular arrangement, and by the end of 3 weeks they show well-developed cross-striations.

3. The new muscle fibres in the gracilis grafts are partly derived from the few surviving old fibres of the graft, and partly by ingrowth from the fibres of the surrounding muscle.

4. If a piece of gracilis muscle is excised and replaced in position at right angles to its original orientation, the regenerated fibres are disposed

transversely, i.e. along the line of the old degenerated fibres. Ingrowing fibres from the surrounding muscle make a right-angled bend as they enter the graft to follow the course of the reorientated old fibres.

5. Following a local crush in the gracilis muscle of a rat, sarcoplasmic outgrowths from the stumps of muscle fibres at the margin of the injury are present on the third day. By the end of the fourth day the young regenerating muscle fibres have crossed the crushed zone.

6. The fascicular pattern of the muscle may become partly reconstituted in the crushed zone in those cases in which perimysial septa have not been completely interrupted, but the constituent fibres of each fasciculus show considerable disorientation.

7. At the end of 3 months, the crushed gracilis muscle is united across the line of the crush injury by interweaving fibres of regenerated muscular tissue. While many of the regenerated fibres appear histologically to have reached maturity, others show wide variation in their size and the degree of their development and differentiation.

8. In two rabbits the tibialis anterior muscle was devascularized and the extent of the resulting ischaemia and necrosis confirmed 2 days later by the intravenous injection of bromo-phenol blue. The muscles were examined 3 and 4 months respectively after operation, and in both cases the necrotic portion of the muscle was found to show regenerated muscular tissue of approximately normal structure. In one case the reconstitution of the muscle was practically complete.

9. The regeneration of muscle fibres does not usually commence until after the first 2 days following injury.

10. No evidence has been found that regenerating muscle fibres arise otherwise than as continuous outgrowths from the stumps of old, pre-existing fibres.

11. In the early stages, regenerating fibres grow out as fine sarcoplasmic strands with pointed tips. Where they meet with obstruction in their growth, the advancing tips become expanded and may form large multi-nucleated masses of sarcoplasm.

12. Regenerating muscle fibres in the rabbit grow at a maximum rate of at least 1–1·5 mm. per day.

13. Following deprivation of blood supply, almost all the endomysial cellular elements as well as the contractile substance of the muscle fibres undergo rapid dissolution.

14. Longitudinal striation of regenerating fibres is often evident from their first appearance. Cross-striation may be well developed by the sixth day of growth, and is sometimes indistinctly evident before this time.

15. Nuclear division in regenerating muscle fibres takes place (at least as a general rule) by amitosis.

16. Short segments of old muscle fibres may give rise to regenerating fibres from both ends simultaneously.

17. In the reconstitution of muscular tissue which has undergone ischaemic necrosis, the ingrowth of regenerating fibres is facilitated and directed by fibroblastic activity. Fibroblasts invade the endomysial spaces in advance of the growing ends of the new fibres, forming new endomysial tubes, and reinforcing or reconstructing the sarcolemmal sheaths around the old necrotic fibres. As the latter are progressively removed by histiocytic activity, they are as quickly followed up by the regenerating fibres which grow down within the newly constructed endomysial tubes. In this manner the original pattern of fibre architecture of the muscle is reproduced in the regenerated tissue.

18. New muscle fibres growing into areas filled with blood clot or serous exudate lose their regular arrangement and become grossly disorientated.

19. Many of the regenerating fibres undergo bifurcation in the process of their growth so that, in the first few weeks at least, the regenerated tissue may contain many more fibres than the original tissue. There is evidence that some of the excess muscle fibres disappear at a later stage of regeneration.

20. There is evidence that the abundant endomysial connective tissue formed in the early stages of the muscle regeneration which occurs in areas of ischaemic necrosis may become reduced almost to normal by the end of 4 months.

I wish to express my thanks to Mr E. Thompson for his expert assistance in the preparation of histological material, and to Mr W. Chesterman for the microphotographs which illustrate this paper.

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## EXPLANATION OF PLATES

### PLATE 1

Fig. 1. Portion of a gracilis graft (R 331) 7 days after implantation. The old fibres are completely necrotic and undergoing fragmentation. Under the action of the fixative, the fibres tend to fracture transversely through planes of cleavage represented by the isotropic discs. Note that the sarcolemmal membranes are preserved intact in many places. The endomysial cellular elements have disappeared. A few leucocytes are seen in the endomysial spaces. ×230.

Fig. 2. Section through the margin of a gracilis graft (R 345) which had been replaced *in situ* at right angles to its original position and left in position for 3 weeks. To the left are seen the longitudinal fibres of the gracilis muscle surrounding the graft. To the right are seen the regenerated fibres in the graft running transversely. Between these areas is the fibroblastic junctional zone between the graft and the surrounding muscle, occupied by strands of newly formed muscle fibres which turn at right angles to invade the graft. ×47.

Fig. 3. Portion of a gracilis graft (R 333) 18 days after implantation. The degenerated fibres have now been replaced by young regenerated fibres with well-developed cross-striations. ×114.

Fig. 4. Section through a crushed gracilis muscle at the site of the crush 4 days after operation (rat A 14). The site of injury is crossed by bundles of fine regenerating fibres which have grown in from the ends of the muscle fibres on either side. Note that the new fibres tend to preserve the fascicular pattern of the muscle. The component fibres of the fasciculi, however, are interwoven with each other. ×58.

Fig. 5. Section through a crushed gracilis muscle at the site of the crush 3 months after operation (rat A 16). Regenerated fibres are still seen at all stages of development from extremely fine strands up to fibres of normal diameter. ×330.

Fig. 6. Section through a gracilis graft 11 days after implantation (R 332). In the centre are seen short segments of surviving old muscle fibres from both ends of which regenerating fibres have grown out on either side. The old fibres show a considerable degree of vacuolization. ×72.

### PLATE 2

Fig. 7. The margin of the crushed zone of a gracilis muscle, 2 days after operation (rat A 13). Extending from the stumps of the muscle fibres are seen the sarcolemmal tubes of the damaged part of the fibres, now filled with large numbers of histiocytes. ×250.

Fig. 8. The first stage in the development of a regenerating fibre from a crushed gracilis muscle 3 days after operation (rat A 17). A nucleated plasmodial outgrowth is seen extending from the end of a pre-existing muscle fibre into the empty portion of its sarcolemmal tube. The stump of the old fibre shows a considerable degree of vacuolization. ×250.

Fig. 9. Section through a crushed gracilis muscle at the site of the crush 3 months after operation (rat A 16). The individual fibres which have regenerated are disposed at all angles and are knitted together by interweaving in a complicated manner. ×95.

Fig. 10. Photograph showing the appearance of the crushed zone in a gracilis muscle immediately after crushing. The muscle fasciculi appear to be completely interrupted. ×4.

Fig. 11. Photograph showing the appearance of the crushed gracilis muscle in rat A 9, 6 weeks after crushing. The region of the crush is indicated by a slight irregularity in the fasciculi which have regenerated. The result in this particular experiment was especially favourable. Sections show that the fibres within each fasciculus have not the regular parallel arrangement of the uninjured part of the muscle. ×4.

Fig. 12. Fibres of gracilis muscle at the margin of the crushed zone 3 months after operation (rat A 16). Note regenerated fibres at all stages of development. ×330.

Fig. 13. Fibres in the crushed zone of gracilis 3 months after operation (rat A 16). The fibres are seen anastomosing with each other. ×330.

### PLATE 3

Fig. 14. Section from a tibialis anterior muscle 8 days after devascularization (R 294). Above are seen the fine strands of regenerating fibres streaming down to the margin of the necrotic zone. Below are the old necrotic fibres, and extending between them is rapidly proliferating fibroblastic tissue in the process of constructing new endomysial sheaths. ×119.

Fig. 15. Section from the tibialis anterior muscle 8 days after devascularization (R 294). The necrotic fibres shown are close to the advancing line of regeneration. Fibroblasts are seen extending through the oedematous exudate in the endomysial spaces, in the process of constructing new endomysial tubes and reinforcing or replacing the old sarcolemmal sheaths. Histiocytes and leucocytes are also present in the fibroblastic tissue. Above and to the left a clump of histiocytes is seen in the substance of an old necrotic fibre. ×250.

Fig. 16. Section from a tibialis anterior muscle at the junction of its upper and middle thirds, 8 days after devascularization (R 294). Above are seen the stumps of pre-existing fibres, and from them regenerating fibres extend down as fine strands of sarcoplasm towards the necrotic portion of the muscle. ×63.

Fig. 17. Section from a tibialis anterior muscle 10 days after devascularization (R 363). The regenerating fibres are seen growing down to replace the old degenerated fibres. The ends of the latter are seen to be capped with clumps of histiocytes in process of removing them by phagocytosis. The new fibres show terminal expansions where they come up against the old fibres. In the section they are separated at their point of contact by a shrinkage space. ×310.

Fig. 18. Section of the lower part of a tibialis anterior muscle 10 days after devascularization (R 359), showing the new regenerated fibres. Cross-striation is evident and most of the nuclei occupy a central position in the young fibres. Note the conspicuous endomysial tubes which are characteristic of regenerated muscular tissue in the early stages and which appear to play an important part in directing the growth of the fibres. The section has been stained with van Gieson's stain. ×230.

Plate 4

Fig. 19. Transverse section through the middle of the lower two-thirds of a normal tibialis anterior muscle from the opposite side of the same animal as that shown in the next illustration (R 329) and photographed at the same magnification. These two microphotographs should be compared in regard to the size of the old and new muscle fibres and the amount of endomysial tissue. ×114.

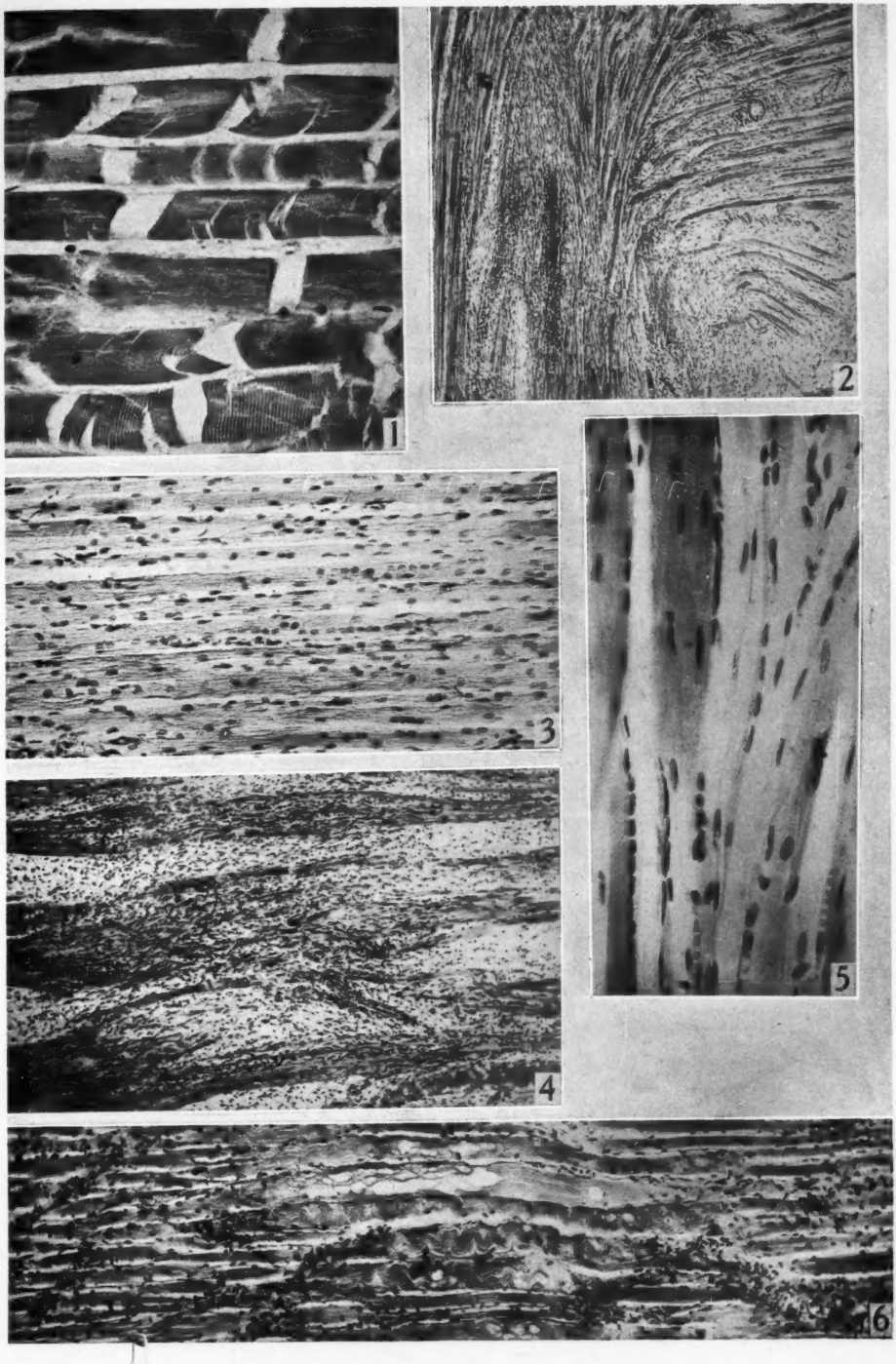
Fig. 20. Transverse section through the middle of the lower two-thirds of a tibialis anterior muscle 21 days after devascularization (R 329). The necrotic muscular tissue has been removed and replaced by regenerating fibres. Note the rounded contour of the new fibres as seen in cross-section, the variation in their size, and the excessive amount of connective tissue. The newly constructed endomysial sheaths surrounding individual fibres are also well shown. ×114.

Fig. 21. Section from the lower part of a tibialis anterior 23 days after devascularization (R 347), showing the newly constructed endomysial tubes which still contain fragments of the contractile substance of the old necrotic fibres. From above, regenerating fibres are seen growing down into the endomysial tubes. Numbers of histiocytes are still to be seen in the field. ×230.

Fig. 22. Section from the lower part of a tibialis anterior 23 days after devascularization (R 347), showing newly constructed endomysial tubes. The remains of the old necrotic muscle fibres have been completely removed in this region and the tubes have not yet been invaded by the regenerating fibres. ×230.

Fig. 23. The stump of a necrotic muscle fibre from a tibialis anterior muscle 8 days after devascularization (R 294). The fibre is undergoing hyaline degeneration, but at the end rounded cells appear to be budding off from it to form detached cellular elements. The fate of these elements is uncertain. ×670.

Fig. 24. Photograph of a tibialis anterior muscle exposed 2 days after devascularization (R 351). The animal had been injected with bromo-phenol blue 10 min. before the photograph was taken. The upper third of the muscle (to the right of the photograph) was stained a deep blue while the lower two-thirds remained unstained. Note the sharp line of demarcation between the two parts of the muscle. This muscle was left undisturbed for 4 months at the end of which time the lower part (which had undergone ischaemic necrosis) had been reconstituted by regeneration of new muscular tissue. ×4 approx.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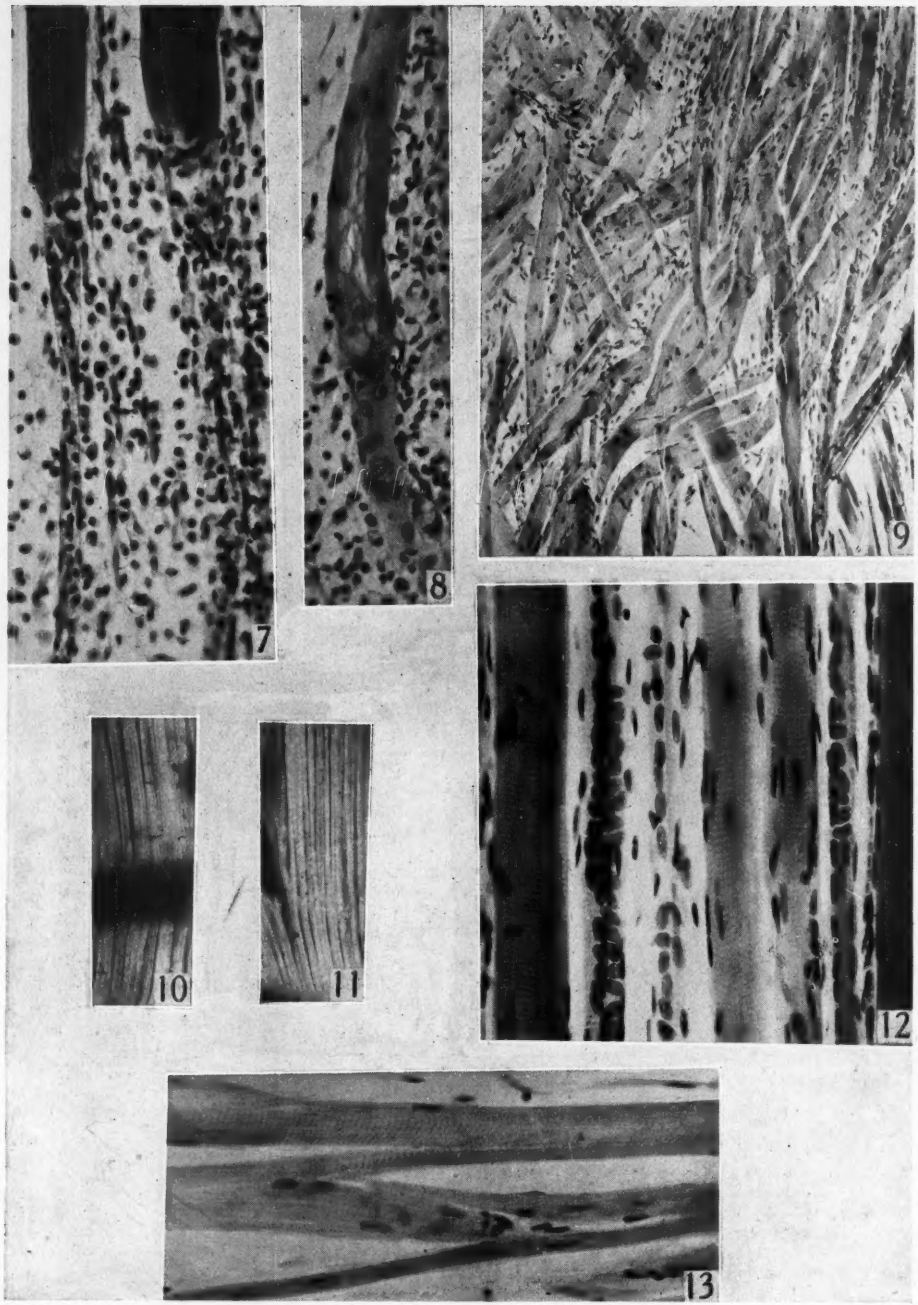
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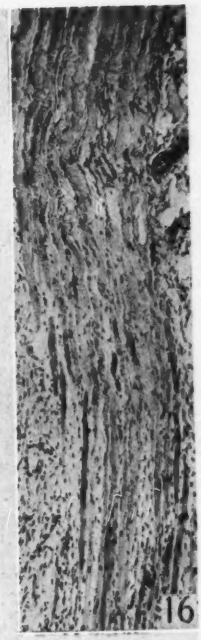
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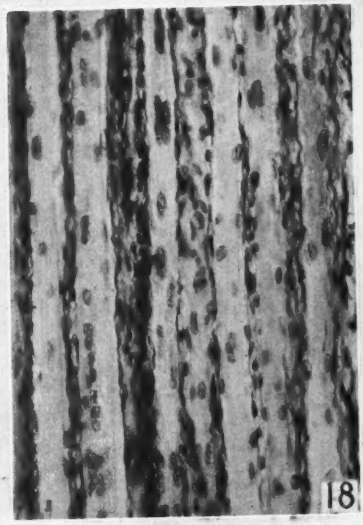
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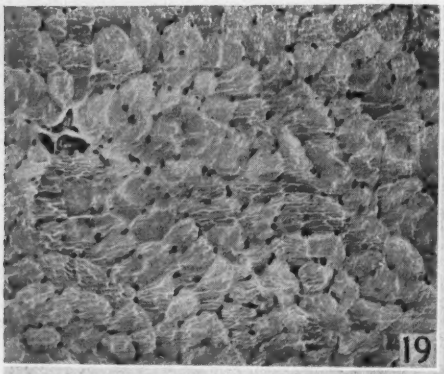
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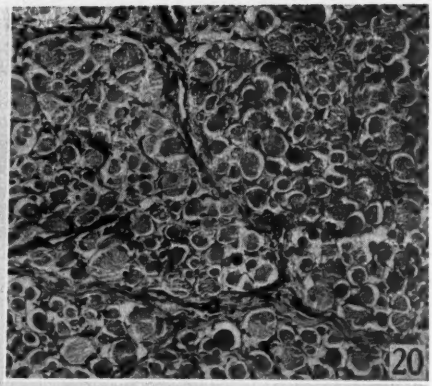
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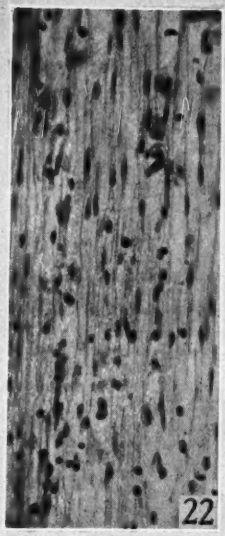
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# QUANTITATIVE HISTOLOGY OF WALLERIAN DEGENERATION

## I. NUCLEAR POPULATION IN RABBIT SCIATIC NERVE

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### INTRODUCTION

The histology of Wallerian degeneration of peripheral nerves has been thoroughly studied in its qualitative aspects (see, for instance, Cajal, 1928; Nageotte, 1932; Weddell & Glees, 1941; Holmes & Young, 1942). In this and subsequent papers our purpose is to provide that more detailed and exact analysis of the process which can only come from quantitative study. We consider this worth attempting not only because Wallerian degeneration is intrinsically an important pathological process. It is also a proliferative phenomenon similar in many respects to the reaction of tissues to local injury. It provides particularly favourable material for experimental analysis of a proliferative process, since it takes place fairly evenly throughout a long stretch of well-demarcated tissue, the peripheral stump of a severed nerve; and the intensity of the reaction is independent of intensity of trauma. Our results may therefore be useful for a comparative survey of proliferation in a variety of tissues.

In this paper we survey the changes in number of nuclei of nerves, excluding the perineurium and epineurium, during Wallerian degeneration, as seen in histological sections. The nuclei of different kinds of cells have, as far as practicable, been analysed separately. We have distinguished between nuclei within the Schwann tubes and nuclei in the endoneurium. The tubal nuclei have been separated into three groups according to size of tube, and the endoneurial nuclei into connective tissue nuclei (fibrocytes and macrophages) and blood vessel nuclei (endothelial and smooth muscle nuclei). The difficult and complicated history of the migratory macrophages cannot be adequately followed in our material. Evidence as to their multiplication and movement can, however, be got from vitally stained animals, and we shall consider this in a subsequent paper. For the present, macrophage and fibrocyte nuclei are not distinguished in the endoneurium, nor macrophage and Schwann nuclei in the Schwann tubes.

### MATERIAL AND METHOD

#### *Material*

We have used exclusively the peroneal and tibial branches of the sciatic nerve in the thigh of rabbits. For our degenerated material we cut the nerve aseptically with sharp scissors at an initial operation, under nembutal and ether anaesthesia, in the thigh at about 1–2 cm. below the level of the third trochanter. When the period of degeneration was to be longer than 100 days, we brought the central stump outside the muscles and sutured it to the under surface of the skin to minimize reinnervation. In the shorter experiments a gap of 1–3 cm. was left between the two stumps. After the required period of degeneration the rabbit was killed and part of the peripheral stump in the thigh removed and fixed. We have not used the proximal centimetre of the peripheral stump, in the neighbourhood of the trauma, in this work. For our undegenerated material we fixed the corresponding region of nerves in unoperated rabbits.

Our material consisted of sixty-five nerves from thirty-three rabbits, studied intensively by differential counts. In the majority of these animals peroneal and tibial nerves of both sides were used, usually cut at different times and then simultaneously fixed, so that comparison of different periods of degeneration could be made under closely similar conditions. Two tibial or two peroneal nerves prepared in this way will be referred to as a *paired experiment*. We have, in addition to this intensively studied material, used a further forty nerves from thirty rabbits to confirm various points.

#### *Histological technique*

Fixation was principally in Susa, though we have also used Bouin, and we found that Bodian's peripheral nerve fixative often gave useful help in differentiating tubal from endoneurial cells because of the severe shrinkage it causes. We used longitudinal and transverse paraffin sections cut at 7 $\mu$. One slide was stained in Heidenhain's haematoxylin and Masson's light green; another slide was usually stained in Mayer's carmalum, which strongly differentiates nuclei from nerve-fibre remnants.

All but four of the nerves of 35 days of degeneration and over, and the majority of nerves of 25 days of degeneration, were fixed and stained by Bodian's method and examined for reinnervation. In almost all of the nerves degenerated 90 days and over a few axons were present, but in no case was reinnervation extensive.

#### *Counting*

Transverse sections (referred to as T.S.) proved much superior to longitudinal ones (referred to as L.S.), both for identification of cell types and for

standardization of nerve volume. Changes in nuclear size however affect T.S. much more seriously than L.S., and we had to make special allowances for them. Our usual procedure has been to count differentially, under an oil-immersion objective, a number of fields selected at random in the T.S. of each nerve. Each field contained 20–200 cells, and the number of fields counted, usually 10–20, varied according to the population. Further supplementary counts were often added, including counts of all nuclei in an entire T.S. A different section was used for each field counted, although variation of equivalent fields from section to section was unimportant. Much more important was randomization of position of the fields in the transverse plane: the 'nerve fibre spectrum' (that is, the relative numbers of fibres of different sizes) varies greatly from place to place in a T.S., and the number of nuclei, at least in the early stages of degeneration, varies with it.

### *Expression of results*

Our results are necessarily expressed in highly summarized form. In the tables which follow, the original counts have been standardized for nerve volume and nuclear length (see next section), and the tibial and peroneal results combined after expressing each as a percentage of the mean population of undegenerated tibials or peroneals respectively. Tibials and peroneals are sufficiently similar in composition to permit this, though in absolute number of nuclei tibials, because of their larger diameter, average throughout degeneration 2·15 times the population of peroneals.

Counts of all nuclei have been done on rather a larger sample than have the differential counts (see Tables 2, 4 and 5). The figures have been made consistent as between the various tables by applying the percentage composition derived from the differential counts to the absolute values of all nuclei. The inconsistency arising from the smaller sample of differential counts is in any case small.

The nerves have been formed into eight groups according to period of degeneration, each group designated by its mean period of degeneration. These groups, and (in brackets) the range of period included in each, are as follows: 0 days (i.e. normal undegenerated nerves); 5 days (5 days only); 10 days (10 and 11 days); 15 days (14–16 days); 25 days (23–26 days); 45 days (35–55 days); 110 days (96–160 days); and 225 days (210–250 days). Nerves intermediate between these groups have, in the interests of brevity, been omitted from the tables, though they are sometimes referred to in the text.

## STANDARDIZATION FOR NERVE SIZE AND NUCLEAR SIZE

If we suppose that changes in the number of nuclei per microscopic field during the course of degeneration indicate accurately changes in the nuclear population of nerve, we make two assumptions: that the volume of the nerve remains constant during degeneration; and that the size of the nuclei also remains constant. Neither assumption is correct.

### *Nerve size*

It is well known that the diameter of a peripheral stump changes during degeneration, at first increasing, then decreasing. Table 1 shows the magnitude of these changes. In the third column the mean cross-sectional areas of fixed and sectioned tibials and peroneals, excluding both perineurium and epineurium, are expressed together as a percentage of that of undegenerated nerves; they are graphed in Fig. 1. These figures give rather a rough indication of the size changes, because considerable variability has been introduced by histological treatment, and peroneals and tibials do not change proportionately.

Table 1. *Changes in nerve size and nuclear length with time of degeneration*

$N$ = number of nerves used for measurements.

| | Nerve size | | Nuclear length | |
|---|---|---|---|---|
| Days of degeneration | $N$ | Mean cross-sectional area as % of 0-day | $N$ | Mean length in $\mu$ |
| (1) | (2) | (3) | (4) | (5) |
| 0 | 14 | 100 | 9 | 12·9 |
| 5 | 6 | 108 | 7 | 11·3 |
| 10 | 5 | 123 | 7 | 9·1 |
| 15 | 4 | 114 | 6 | 8·9 |
| 25 | 13 | 118 | 9 | 8·6 |
| 45 | 7 | 96 | 9 | 10·2 |
| 110 | 9 | 97 | 8 | 11·2 |
| 225 | 6 | 73 | 6 | 12·0 |

In order to allow for these changes in size of nerve, and for the additional variation produced by histological treatment, our index of nuclear population has been the number of nuclei in a complete transverse section of the nerve. In many cases we have directly counted all the nuclei in a section. In others, where a complete count was impracticable, we have estimated the number from sample fields and the area of the section.

### *Nuclear size*

In a section the objects counted as nuclei vary from entire nuclei to minute terminal fragments. It is impracticable to differentiate them, and all must be counted alike. The longer the nuclei the more of them will be represented in each transverse section. Nuclear length certainly shortens during the early cell-division phase of Wallerian degeneration, and subsequently lengthens during the shrinkage in diameter of the nerve. Comparisons of nuclear population at different stages of de-

generation demand therefore a correction of counts for change in nuclear length.

The method we have used for making the necessary correction is to measure nuclear length in longitudinal sections, taking the mean of 50 or 100 random measurements on each nerve. All T.S. nuclear counts of degenerated nerves are then standardized to the value they would have if the nuclei were the same length as those in undegenerated nerves. This is done by multiplying the crude count by $(7+a)/(7+b)$, 7 being the thickness in $\mu$ of the T.S. counted, $a$ the mean L.S. nuclear length in $\mu$ of undegenerated nerves, and $b$ the mean L.S. nuclear length in $\mu$ of the degenerated nerve in question.

Mean nuclear lengths of all nuclei (without consideration of cell-type) obtained from L.S. measurements are given in Table 1, and graphed in Fig. 1.

## CHANGES IN TOTAL NUCLEAR POPULATION

Table 2 shows the changes in population of all nuclei with time of degeneration. The mean number of

Table 2. *Changes in total nuclear population with time of degeneration*

| Days of degeneration | No. of nerves counted | Mean population as % of 0-day | Standard error |
|---|---|---|---|
| (1) | (2) | (3) | (4) |
| 0 | 15 | 100 | 2·4 |
| 5 | 5 | 163 | 8·5 |
| 10 | 5 | 390 | 23 |
| 15 | 5 | 635 | 35 |
| 25 | 12 | 840 | 66 |
| 45 | 8 | 600 | 56 |
| 110 | 7 | 540 | 41 |
| 225 | 8 | 500 | 26 |

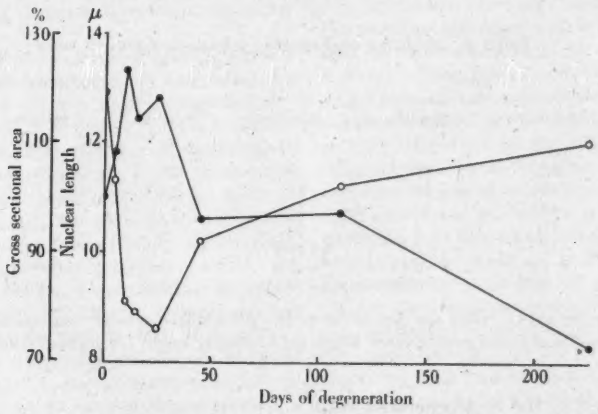


Fig. 1. Mean cross-sectional area of nerves expressed as percentage of that of undegenerated nerve ● and mean nuclear length in $\mu$ ○ at different times of degeneration.

Clearly the mean L.S. length will be less than the true nuclear length because we cannot differentiate whole nuclei from shorter nuclear fragments in a section. We estimate the maximum underestimation at 10%, but must defer justification of this statement to a subsequent publication. The method is quite accurate enough for most of our material. But at 225 days of degeneration, and to some extent at 110 days, an error from changing shape of nucleus becomes serious because of the sinuosity of many of the nuclei, which produces a high proportion of short fragments in L.S. The length given in Table 1 for 225 days of degeneration is an estimate based on selection of the straighter nuclei. It is, nevertheless, probably too low. The largest correction for nuclear length occurs at 25 days of degeneration. In these nerves the crude count is 23% less than the population standardized to 0-day nuclear length. The correction is therefore important.

nuclei in a complete T.S. at different times of degeneration, corrected for changes of nuclear length, has been expressed as a percentage of the mean population of undegenerated nerves. The data are shown graphically in Fig. 2.

The nuclear population increases rapidly during the first 25 days, and then falls. We will consider these changes in two sections.

### *The population increase*

There is no significant change in population during the first 3 days of degeneration. The small group of nerves (not shown in the table and figure) at 3 days averages 103% of the undegenerated group; and two paired experiments show the 3-day nerve as 108% of the undegenerated. Rapid multiplication then starts, and by 25 days the population has reached a value averaging 8·4 times that of the undegenerated group. In the case of an average

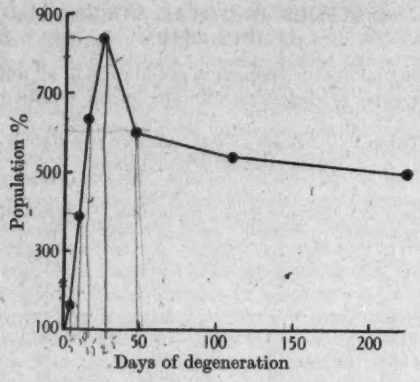


Fig. 2. Mean total population of nuclei of nerves at different times of degeneration, expressed as percentage of that of undegenerated nerve.

average number of nuclei added per day during various periods of degeneration. Since any initial value would be somewhat arbitrary, it is expressed for convenience as the daily increment to an initial 100 nuclei of the undegenerated nerve. It shows that the period of steepest average rise of the growth curve is 10–15 days. At this time an average peroneal is adding nuclei at 10 per day per $\mu$ of its length. Before and after this period the daily increment is less, though not very much so. The growth curve is therefore sigmoid.

Presumably the number of nuclei present at a given time in a nerve is of some importance in determining the additive growth rate at that time. The appropriate measure of increase relative to population present is the *specific growth rate*, which is given as an average figure for each of the various periods of degeneration in Table 3, column 3, and shown graphically in Fig. 3. This is the average

Table 3. *Additive and specific population growth rates*

| Period of degeneration days | All nuclei | | Tubal nuclei | | Connective tissue nuclei | |
|---|---|---|---|---|---|---|
| | Additive | Specific | Additive | Specific | Additive | Specific |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| 3–5 | 32 | 24 | 19 | 28 | 13 | 24 |
| 5–10 | 45 | 18 | 35 | 22 | 11 | 12 |
| 10–15 | 49 | 10 | 42 | 11 | 7 | 5 |
| 15–25 | 21 | 3 | 20 | 4 | 0·8 | 0·8 |
| 25–45 | −12 | −1·7 | −10·4 | −1·9 | −1·6 | −1·2 |
| 45–110 | − 1·0 | −0·16 | − 0·9 | −0·21 | −0·05 | −0·04 |
| 110–225 | − 0·35 | −0·07 | − 0·31 | −0·08 | −0·02 | −0·01 |

The additive and specific growth rates are defined on p. 40. All additive growth rates are referred to an initial 100 nuclei *of all kinds*. Blood-vessel nuclei growth rates, which are extremely small, are omitted. There is no growth between 0 and 3 days.

peroneal nerve, which in the undegenerated state has nuclei distributed at the rate of 20 per $\mu$ of length of the nerve, this means the addition of 150 nuclei per $\mu$ of length. (A T.S. count suggests a density of nuclei more than twice as high as these figures. That is because most nuclei are represented in more than one section.)

The standard errors of the means at different times of degeneration are given in column 4 of Table 2. All the means covering the increase of populations are significantly different from each other by the $t$ test, except that the 15-day mean is not significantly different from the 25-day mean. Nevertheless, we are confident that the increase of population continues between 15 and 25 days, since two paired experiments showed 25-day populations averaging 1·3 times the 15-day population (the grouped data of Table 2 also give a corresponding increase of 1·3 times); and mitoses are frequent beyond 15 days of degeneration.

In Table 3, columns 2 and 3, the increase is presented in terms of growth rates. Column 2 of this table shows the *additive growth rate*, i.e. the

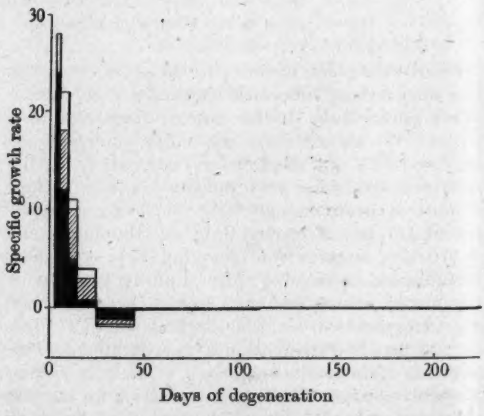


Fig. 3. Specific growth rates of all nuclei ▨, tubal nuclei □, and connective tissue nuclei ■ at different times of degeneration.

number of nuclei added per day per 100 cells present, obtained from the formula

$$100\,(\log_e N_2 - \log_e N_1)/t_2 - t_1 = R,$$

where $N_1$ and $N_2$ are nuclear populations at $t_1$ and $t_2$ days of degeneration respectively, and $R$ is the specific growth rate. (The specific growth rate is the 'relative growth rate' of Fisher (1938, p. 29); see Medawar (1940) for the change in nomenclature.)

The specific growth rates show that multiplication of nuclear population is most rapid early in degeneration and declines in rate as degeneration proceeds. This conclusion can also be derived from a considerably larger sample of nerves (thirty-nine nerves between 0 and 10 days, including a large 7-day group) for which we have counts of nuclei per field in L.S. Specific growth rates from this data (unstandardized for nerve or nuclear size) show the same decline during degeneration from an initial maximum.

If an initial phase of accelerating specific growth rate exists, it cannot be detected in our data. The specific growth rate at 3–5 days is significantly higher than the rate at 5–10 days by $t$ test. It should be noted, of course, that the *day* of most rapid growth rate, additive or specific, is not necessarily within the 2- or 5-day period of most rapid average growth, which is all that our data give us. We discuss these growth rates further when we consider the different cell-types separately.

The number of nuclei per unit volume of nerve may be physiologically important. It increases by about 7 times during the first 25 days of degeneration. This means an average increase from roughly 43,000 nuclei per mm.$^3$ in the undegenerated nerve to 330,000 per mm.$^3$ at 25 days of degeneration.

### *The population decrease*

At 25 days, according to our data, the peak of population is reached. We should emphasize, however, that 25 days is not necessarily the true average peak of population, since we have studied nerves at discrete intervals of degeneration and do not accurately know what happens between them. We believe, however, that the curve is likely to be nearly flat between 20 and 30 days, judging roughly by mitosis and nuclear degeneration, and that 25 days is not likely to be far from the average time of maximum population.

The population built up during the first 25 days of degeneration does not persist at that size. We have nerves up to 225 days of degeneration, and during that time the population declines to about 60 % of the peak value. This population is equivalent to that of a nerve of 12 days of degeneration. The extent of the decrease receives additional support from three paired experiments. In these the population of the 225-day peroneal was respectively 66, 55 and 53 %, an average of 58 %, of its contralateral 25-day peroneal. The change in a typical peroneal represents a decline from 170 to 100 nuclei per $\mu$ of nerve length.

The loss of cells is statistically significant. Comparing means of peroneals of 25 days of degeneration with those of 100 days and over, a $t$ test shows that the probability that both samples come from the same population is only 0·006 ($t = 3{\cdot}2$, $N = 18$). This is without any correction for nuclear length, which must increase the significance, since the nuclei elongate after 25 days.

The curve in Fig. 2 suggests that the greater part of the decrease of nuclear population occurs between 25 and 45 days. This is also shown in Table 3, which gives additive and specific growth rates (negative during the decrease of population).

It is possible that the extreme concentration of nuclear loss within this period is a sampling error. The decrease is after all a small one compared with the previous increases, and it is too much to expect that our material should be accurate within such narrow limits. The 40–50-day nerves are of unusually small size (see Table 1), and their mean number of nuclei per field in T.S. is actually slightly smaller than that of the 100-day group. All this suggests that the 45-day mean is too low, and therefore that the loss of nuclei is more evenly distributed.

Nevertheless, we believe our data make it very probable that the most important loss does occur soon after the peak of population. This view is supported by estimates of specific growth rates (% per day) from paired experiments, which should not be affected by sampling error in total population (the pairs were 25 and 50, 50 and 100, 100 and 250 days; we had two of each of these pairs). These growth rates are, for the 25–45-day period, $-0{\cdot}8$; and for the 45–225-day period, $-0{\cdot}15$. Additional support comes from the accurately determined percentage composition of nerves which is also independent of sampling error in total population. Loss of tubal nuclei makes much the biggest contribution to the population decrease, and the percentage which tubal nuclei form of all nuclei declines much more rapidly between 25 and 45 days than between 45 and 225 days (see Table 4, column 4). This is true regardless of the period to which we allocate the small loss of endoneurial nuclei.

We cannot unfortunately give statistical proof that the nuclear loss continues beyond 45 days. But the general trend of all our data suggests that it does. The most likely supposition as to the course of the population curve beyond 25 days of degeneration is as follows: the population falls, as it rises, with a sigmoid curve; the inflexion of the curve is very early in the process; but the fall continues at diminishing rate throughout the period we have studied.

The density of population (number of nuclei per

unit volume) is also maximal at 25 days, but the decline thereafter is slight (density at 225 days is about 90 % of that at 25 days). The large decrease in total population is almost compensated by the shrinkage of the whole nerve.

### *Variability*

The populations of the undegenerated nerves have a relatively low dispersal about their mean (coefficient of variation $9{\cdot}3 \pm 1{\cdot}7$), while at 25 days of degeneration the dispersal is high (coefficient of variation $27 \pm 6$) and is similar at later stages of degeneration. The proliferative response to degeneration seems therefore to vary widely from rabbit to rabbit.

### *Peroneal-tibial comparison*

Throughout degeneration the peroneal nerves have a slightly but constantly higher density of nuclear population per unit volume than the tibial nerves, averaging 13 % more. Further, when the total nuclear populations given in Table 2, column 3, are expressed separately for tibials and peroneals, the 0-day mean population in both cases being taken as 100, the peroneals average about 9 % higher than the tibials, indicating possibly a very slightly greater response to degeneration in the peroneals. Clearly, it is hardly to be supposed that the two nerves are identical in their behaviour, but the differences between them are quite insufficient to necessitate their separate treatment. The slight difference between the means of the two branches exaggerates the standard errors given in Table 2, column 4.

## CHANGES IN TUBAL NUCLEAR POPULATION

### *Identification and standardization*

In this section we consider those nuclei which lie inside the *Schwann tubes* of Holmes & Young (1942). Most of these tubes can be easily recognized in transverse section. But a nucleus substantially fills a tube of less than 3–4 $\mu$ diameter, swelling it out if it is elsewhere narrower than the nucleus. Such a tightly invested nucleus in the undegenerated and early degenerated nerve is often not easy to distinguish from a longitudinally oriented endoneurial nucleus. As endoneurial oedema increases during degeneration, however, endoneurial nuclei become increasingly distinct, and by 10 days little uncertainty exists. A sure way of establishing whether a nucleus is tubal or not is to trace it through serial sections. This is impracticable in large-scale counting, but samples investigated in this way have given results reasonably concordant with our ordinary counts. Nevertheless, the margin of error here, and particularly with regard to the 0-day nerve, is greater than elsewhere, and we have accordingly been cautious in drawing conclusions about the early stages of degeneration.

As is well known, the largest tubes become invaded by macrophages during degeneration (see Doinikow, 1913). Intravital staining with trypan blue shows that this invasion begins at 5 days of degeneration.

Table 4. *Changes in population of tubal nuclei with time of degeneration*

| | | Mean tubal population | | % distribution of tubal nuclei | | |
|---|---|---|---|---|---|---|
| Days of degeneration | No. of nerves counted | as % of 0-day | as % of all nuclei | Large tube | Medium tube | Small tube |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| 0 | 8 | 100 | 50 | 16 | 21 | 63 |
| 5 | 5 | 174 | 54 | 8 | 17 | 75 |
| 10 | 5 | 520 | 66 | 38 | 21 | 41 |
| 15 | 5 | 940 | 73 | 37 | 29 | 34 |
| 25 | 9 | 1340 | 79 | 34 | 36 | 30 |
| 45 | 7 | 920 | 76 | 25 | — | — |
| 110 | 9 | 800 | 74 | 14 | — | — |
| 225 | 8 | 730 | 73 | 4 | — | — |

The immigration of macrophages, and possibly a later emigration, must be taken into account in assessing the tubal population changes. Morphologically, however, we find it impossible to distinguish tubal macrophages from Schwann cells in T.S., and we can only make general allowance for these migrations until our investigations of trypan blue material are complete.

The figures of tubal population have been standardized for nuclear length to make them consistent with the rest of the figures we quote. This standardization is not, however, based on measurements of tubal nuclei, but on the average nuclear length of all cells in the nerve (excluding blood-vessel cells). Such rough standardization is sufficient for our purposes, though it introduces some obvious inaccuracies which will be referred to when necessary.

Table 4, column 3, shows the mean number of tubal nuclei in complete T.S. at different times of degeneration standardized approximately for nuclear length, and expressed as a percentage of their mean population in undegenerated nerves.

The data are shown graphically in Fig. 4. The population of tubal nuclei shows roughly the same changes as the population of all nuclei (Fig. 2)—a rise to a peak at 25 days of degeneration, with subsequent decline.

*The increase of population of tubal nuclei*

As with all cells combined (see p. 39), there is no detectable change in population during the first 3 days of degeneration. This is followed by a rapid increase which brings the population to a maximum averaging approximately 13 times the original at about 25 days of degeneration. The relation of this increase to that of the other (endoneurial) nuclei of the nerve can be seen in column 4 of Table 4 and in

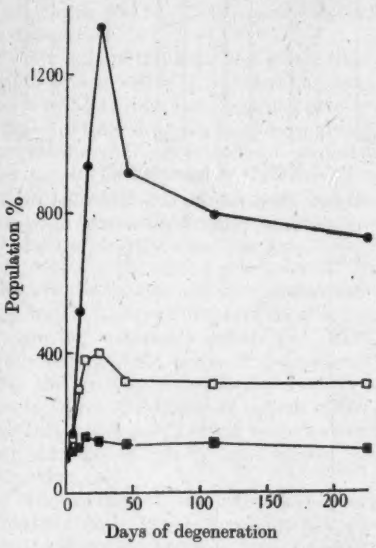


Fig. 4. Mean populations of tubal ●, connective tissue □, and blood-vessel nuclei ■ in nerves at different times of degeneration, expressed as percentages of their respective populations in undegenerated nerve.

Fig. 5, which gives the percentage of all nuclei of the nerve which are intratubal. This percentage increases from 50 in the undegenerated nerve to 79 at 25 days of degeneration. Tubal cells therefore increase much faster than endoneurial cells, though between 3 and 5 days their superiority is not so well marked.

Growth rates of tubal nuclear population are given in Table 3, columns 4 and 5. They show the same trends as those of all nuclei combined. The additive rate given is the daily increment of tubal cells to an initial 100 nuclei *of all types* in the undegenerated nerve. It is thus referred to the same initial population as the additive growth rate of all nuclei, and also that of connective tissue nuclei given in this table. The large proportion of the total increment which is due to the tubal cells is evident by comparing columns 2 and 4. The 3–5-day specific growth rate of tubal nuclei may not really be higher than that of 5–10 days, because of the difficulty over identification; but it is likely to be at least as high. In relation to the whole tubal population, our preliminary information suggests that the macrophage invasion, which is confined to the larger tubes, is so small that it can be safely neglected in assessing growth rates. Correspondingly, any early emigration from the Schwann tubes during this period (Cajal, 1928; Nageotte, 1932) can also be neglected.

*The decrease of population of tubal nuclei*

The decline in population of tubal cells after 25 days of degeneration is greater than that of all cells. The tubal population at 225 days of degeneration is only 55% of the tubal population at 25 days

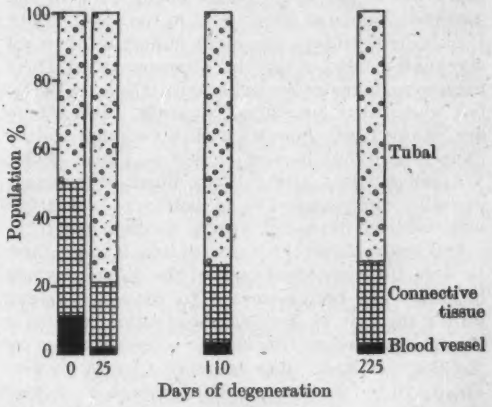


Fig. 5. Percentage composition of nuclear population of nerves at different times of degeneration.

(Table 4, column 3), and during this period the fraction of all nuclei constituted by the tubal nuclei falls from 79 to 73% (Table 4, column 4, and Fig. 5).

The growth rates for tubal nuclei in Table 3 indicate that the most rapid decline is between 25 and 45 days. This we believe to be true, but the extent of the decline is probably exaggerated by sampling error. The arguments put forward for this view with reference to all nuclei on p. 41 hold also for tubal nuclei.

There is no sign that the loss of tubal cells is in any degree due to an emigration of macrophages into the endoneurium, unless such emigrants perish immediately they leave the tubes. The endoneurial population certainly does not rise as the tubal population falls.

The greater part of the loss of tubal cells must be due to degeneration of Schwann cells, even if all the tubal macrophages degenerate in the tubes. This

can be appreciated from Table 6, which is expressed in absolute figures. The loss of all tubal cells (column 3) between 25 and 225 days considerably exceeds the total population of the large tubes at 25 days (column 4); and only a proportion, whose maximum we provisionally place at 25%, of the large tube nuclei can be macrophage nuclei.

*Tubal population and tube size*

We have attempted a rough analysis of tubal population in terms of size of tube. We counted separately the tubal cells at different stages of degeneration (*a*) in 'large' tubes (larger than 9 $\mu$ diameter), (*b*) in 'medium' tubes (4–9 $\mu$ diameter), (*c*) in 'small' tubes (smaller than 4 $\mu$ diameter). Large tubes, and probably medium tubes, contain only myelinated fibres; small tubes both myelinated and unmyelinated. From the analysis of Gutmann & Sanders (1943) rather less than 20% of myelinated fibres fall in the large group, about 30% in the medium group, and about 50% in the small group. Unmyelinated fibres are more numerous than all myelinated ones put together (Ranson, 1912). Their maximum diameter is roughly 1–2 $\mu$ (Duncan, 1934); but while many are single (Nageotte, 1915) others are grouped into bundles with a common sheath (Nageotte, 1932; Ranson, Foley & Alpert, 1933; Weddell & Glees, 1941). These bundles, however, probably rarely exceed 4 $\mu$ in diameter, so that few unmyelinated fibres fall into our medium group.

Our analysis into three sizes of tube is rough, first because the number of each of the different kinds of tubes has been assumed to remain constant during the first 25 days of degeneration, though a change does occur. This change is chiefly a loss by shrinkage of large tubes amounting by 25 days to about 20% of that of undegenerated nerves. Secondly, it is rough because considerable differences in change of nuclear length according to tube size have been neglected. The larger the tube the greater appears to be the decrease in nuclear length, and therefore the large tube nuclei are underestimated relative to the small. Because of the approximate nature of the analysis, we have not tabulated our data on tube size in full.

The percentage distribution of the tubal nuclei among these different classes of tube is shown in Table 4, columns 5–7 and Fig. 6. Fig. 7 shows the changes undergone during the first 25 days of degeneration by populations of the nuclei of the three kinds of tube expressed as percentages of their respective populations in undegenerated nerves. These percentages are derived from Table 6.

It is clear that the population changes of the different tubes have different time relations and different magnitudes. Our main conclusions about tube size in relation to nuclear population are:

(i) The small tube nuclei have a very high specific growth rate in the 3–5-day period, and the additive growth rate is maximal during this period. After this initial burst of activity, moderate multiplication continues until 15 days and then dies away.

(ii) The medium tube nuclei show a continuously fairly high specific growth rate between 3 and 15 days. Its maximum is probably a little later than that of the small tube nuclei; it falls off after 15 days. The additive growth rate rises to a maximum in the 10–15-day period, and then declines again.

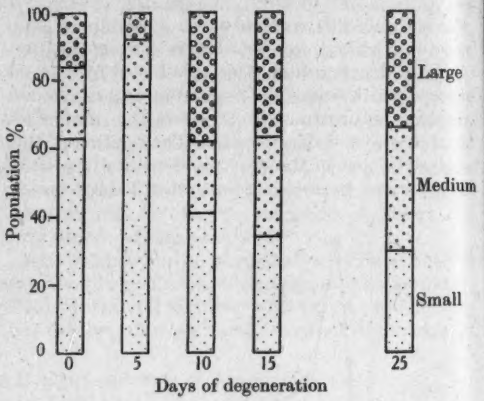


Fig. 6. Percentage composition of tubal nuclear population of nerves at different times of degeneration up to 25 days.

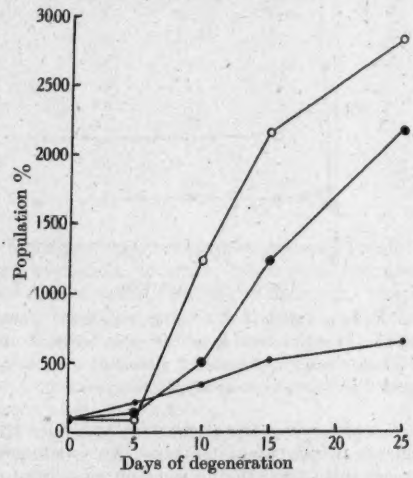


Fig. 7. Mean populations of large ○, medium ●, and small tube • nuclei in nerves at different times of degeneration up to 25 days, expressed as percentages of their respective populations in undegenerated nerve.

(iii) The large tubes show an initial burst of activity like the small tubes, but it starts about 2 days later. Between 3 and 5 days there is an apparent absolute loss of these nuclei, due to the

extreme shortening they undergo by collapse into the interior of the tubes. Between 5 and 10 days, starting probably on the 6th day, there is an extremely rapid multiplication which causes the large tube nuclei to increase from 8 to 38 % of all tubal nuclei (Fig. 6). Even when allowance is made for immigration of wandering cells by supposing that 25 % of this increase is so caused, the specific growth rate over this period remains the highest for any cell-type at any period of degeneration. The additive growth rate for these nuclei is also probably maximal during this period. After 10 days the specific growth rate falls sharply, even when allowance is made for the loss of large tubes through shrinkage. But, because of the large population built up, the additive growth rate remains almost as high during the 10–15-day period as during the 5–10-day period.

(iv) The larger the tube the greater the multiplication of cells within it. The specific growth rates between 0 and 15 days (which period gives a greater constancy of tube size than 0–25 days, though a loss of large tubes has nevertheless occurred) are, in % per day, 26 for large tubes, 21 for medium tubes and 14 for small tubes. If it is supposed that 25 % of the large tube cells are immigrant macrophages at 15 days of degeneration, the large tube specific growth rate for the period would be 23. The large tubes have the highest additive growth rate during this period (0–15 days), while the medium and small tubes are approximately equal. The result of the relation between tube size and specific growth rate is that the nuclear population, which in the undegenerated nerve is sparse in regions of large tubes and denser in regions of small, becomes much more evenly distributed by the end of the period of proliferation.

(v) After 25 days the rapid decrease of percentage of large tube nuclei (Table 4, column 5) is accounted for by the shrinkage of tubes, large tubes becoming medium and medium small. When the large tube population is expressed as nuclei per tube, its decline in numbers is at approximately the same rate as that of all tubal nuclei and is sharpest between 25 and 45 days. The complications of shrinkage during this period are such as to preclude reliable conclusions about small and medium tube nuclei, and they are therefore not treated separately.

(vi) The number of large tube nuclei per unit volume is consistently a little higher (average 14 %) in tibials than in peroneals. This presumably reflects a slight difference in 'fibre spectrum'.

The time relations of nuclear population increase in the different tubes ((i) and (iii) above) will probably be found to be correlated with the time of onset and rate of progress of degeneration in each of the fibre size groups, which is known to differ considerably. Unfortunately, the literature is very confused on these points, owing to lack of definition as to criteria of time and rate, and to absence of statistical treatment. The work of Weddell & Glees (1941) indicates that in general the smaller the fibre the earlier the onset of degeneration. The earliest of all to degenerate are the majority of the non-myelinated fibres (Ranson, 1912). According to our results, specific growth rates of tubal nuclear population have an earlier maximum the smaller the tube size group, and so parallel the time of onset of degeneration.

## CHANGES IN NUCLEAR POPULATION OF ENDONEURIAL CONNECTIVE TISSUE

### *Identification and standardization*

In the endoneurium are fibrocytes, macrophages, and blood vessels with their constituent cells. In this section we discuss the nuclei of fibrocytes and macrophages, including those 'adventitial' cells closely attached to the blood vessels.

In an ordinary transverse section we are unable to distinguish macrophages from fibrocytes with sufficient reliability to separate them; and in longitudinal section we are unable to be sure in every case when a cell is intratubal or endoneurial. We have therefore had to undertake a special analysis of the macrophages, using vitally stained rabbits, which will be the subject of a future paper. For the moment we treat fibrocytes and macrophages as a single 'connective tissue' group.

Endoneurial nuclei are not easily distinguished from Schwann cell nuclei of the small tubes in an undegenerated nerve. As degeneration proceeds, endoneurial oedema soon makes identification much easier.

Two further sources of inaccuracy in assessing changes of nuclear population exist for the endoneurium as for tubal cells. They are: (i) migrations of macrophages, and (ii) inadequate standardization for nuclear length, this standardization being based on the average of tubal and endoneurial nuclear length. These will be discussed as necessary.

Table 5, column 3, shows the mean number of endoneurial connective tissue nuclei in a complete T.S., standardized approximately for nuclear length, and expressed as a percentage of that of undegenerated nerve. The data are shown graphically in Fig. 4.

### *The increase of population of connective tissue nuclei*

As usual, there is no detectable change in population during the first 3 days. The increase which follows brings the population by 25 days of degeneration to about 4 times its original figure. This is, of course, a much slower increase than that of the tubal nuclei, and consequently, as shown in Table 5, column 4, and Fig. 5, the percentage of all nuclei of the nerve formed by the endoneurial connective

tissue group diminishes from 39 at 0 days to 19 at 25 days.

Growth rates are given in Table 3 and Fig. 3. Here it is probable, in spite of the difficulty of identification during the early period, that the specific growth rate is maximal in the period 3–5 days, and declines steeply thereafter. The additive growth rate is at least as high in the 3–5-day as in the 5–10-day period, and perhaps declines throughout the first 25 days, unlike that of the tubal nuclei. It will be noted that in the initial period the endoneurial connective tissue nuclei increase almost as fast as do the tubal nuclei, but subsequently lag far behind. It is probable, however, that connective tissue growth is underestimated as compared with tubal during the period 15–25 days. Many of the connective tissue nuclei are at this time shortening by rounding off, while some tubal nuclei are probably elongating. The application of the same correction to both types of cell probably therefore distorts the true picture.

The connective tissue specific growth rates, regarded as a measure of the multiplication within the endoneurium, are underestimated because of the migration of macrophages into the large tubes. The most liberal allowance for this (assuming 25 % of large tube cells are immigrant macrophages) does not alter the conclusion that the growth rate declines from a maximum at 3–5 days, and is at all times less than the tubal growth rate.

The overall multiplication of the connective tissue is in fact less than that of any of the three tubal types analysed above, even when allowance is made for the migration of macrophages into the large tubes.

*The decrease of population of connective tissue*

The connective tissue population declines after 25 days, but much less than does the tubal. At 225 days the connective tissue population is 75 % of its 25-day population (Table 5, column 3, and Fig. 4), and during this period the fraction of all nuclei constituted by the connective tissue rises from 19 to 24 % (Table 5, column 4, and Fig. 5). The decrease in connective tissue nuclei is, however, probably overestimated, because the average lengthening of all nuclei during this period seems to be mainly due to lengthening of tubal nuclei, while the correction for nuclear length assumes that the connective tissue nuclei lengthen the same amount as the tubal. This source of error is insignificant for the tubal nuclei, since they are in a large majority in the nerve. Supposing that no lengthening of connective tissue nuclei occurred (which is not true), they would by 225 days fall to 82 % of their 25-day value. By *t* test, pooling the 15–25-day data on the one hand and the 45–225-day data on the other, and assuming nuclear length constant throughout, the fall in connective tissue nuclear population is of borderline significance ($t=2{\cdot}3$, $N=31$, $P=0{\cdot}03$).

Table 5. *Changes in population of endoneurial nuclei with time of degeneration*

| | | Connective tissue nuclei Mean population | | Blood vessel nuclei Mean population | | |
|---|---|---|---|---|---|---|
| Days of degeneration | No. of nerves counted | as % of 0-day | as % of all nuclei | as % of 0-day | as % of endoneurial nuclei | as % of all nuclei |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| 0 | 8 | 100 | 39 | 100 | 22 | 11 |
| 5 | 5 | 161 | 38 | 119 | 17 | 8 |
| 10 | 5 | 297 | 30 | 127 | 11 | 4 |
| 15 | 5 | 380 | 24 | 157 | 10 | 3 |
| 25 | 9 | 410 | 19 | 146 | 9 | 2 |
| 45 | 7 | 320 | 21 | 135 | 11 | 3 |
| 110 | 9 | 312 | 23 | 140 | 11 | 3 |
| 225 | 8 | 308 | 24 | 121 | 10 | 3 |

The fall in connective tissue population makes it very unlikely that all the tubal macrophages leave the tubes and enter the endoneurium again during this period, unless they degenerate as soon as they reach it.

The growth rates given in Table 3 show as usual a relatively pronounced fall in the period 25–45 days, and a very slight fall thereafter. We have previously discussed the probability that the sudden decrease of all nuclei in this period may be exaggerated by, though not entirely produced by, sampling error; and the same arguments applied to tubal nuclei. With reference to the connective tissue nuclei, we cannot be sure that the greater part of the decrease between 25 and 45 days is not due to sampling error, and that the small loss of nuclei which probably occurs between 25 and 225 days is not fairly evenly distributed over that period. Two paired experiments, each of 25 and 45 days, showed no difference between populations of connective tissue nuclei at the two times. The question is one for further study.

## CHANGES IN POPULATION OF BLOOD-VESSEL NUCLEI

### *Identification and standardization*

Terminal arterioles, venules and capillaries occur in the endoneurium, in a plexus the meshes of which are chiefly longitudinal. In this section we are only concerned with endothelial and smooth-muscle nuclei, and of these the former far outnumber the latter. The connective tissue cells which occur in association with the blood vessels cannot be sharply distinguished from the rest of the endoneurial connective tissue, and have been counted along with it.

Blood-vessel nuclei occur in much smaller numbers than the nuclei we have so far dealt with; and instead of being fairly evenly distributed, they are arranged in discrete groups, which leads to great variability in the number per field. Correspondingly the counts are less reliable than those of other cell-types.

Unlike all other cells, endothelial nuclei do not shorten during the early stages of degeneration, but, on the contrary, lengthen a little. They have therefore required separate standardization for length, which has been done by measuring them in longitudinal section. Mean lengths in $\mu$ (in brackets the number of nerves measured) were: 0 days, 13·5 (6); 5–25 days, 14·5 (12) (no significant change during this period); 100 days, 14·1 (5). 'Lengths' of smooth muscle nuclei (which are mainly extended transversely to the nerve) have been neglected.

### *Number of blood vessels*

There is no significant change in the number of blood vessels throughout degeneration, although there is considerable random variation. There are about 100 blood vessels, almost all cut transversely, per mm.$^2$ of section.

In the undegenerated nerve each $\mu$ of blood-vessel length supplies about 0·4 cell, at 25 days of degeneration about 3·5 cells, and at 225 days about 2 cells.

### *Changes of nuclear population of blood vessels*

In Table 5, column 5, we give the population of blood-vessel nuclei at different times of degeneration, standardized for nuclear length. They are expressed as a percentage of the mean number in undegenerated nerves, and are shown graphically in Fig. 4. The data suggest an increase to about $1\frac{1}{2}$ times the initial value by 25 days of degeneration (though the maximum may be reached earlier than this), and there is apparently a subsequent decline. The increase is very much smaller than the average for all nuclei, so that the percentage of all nuclei formed by blood-vessel nuclei drops from 11 in the undegenerated nerve to 2 at 25 days (Table 5, column 7, Fig. 5). Similarly, the rest of the endoneurial nuclei increase much faster than the blood-vessel nuclei (Table 5, column 6). The decrease after 25 days is smaller, but not much so, than the average of all cells and of the endoneurium.

The growth rates of blood-vessel nuclei are not tabulated, since they are not very reliable.

The increase in blood-vessel nuclei in early degeneration is substantiated by numerous paired experiments. For instance, a 0- and 23-day pair showed an increase of 46%. The later decline receives some support from four paired experiments, each of 25 and 225 days. Three of these show considerably fewer nuclei at 225 days, while the fourth shows no significant difference between the pairs. These four 225-day nerves average 80% of their 25-day contralaterals.

The evidence seems to us satisfactory that an increase in the number of blood-vessel nuclei occurs. Doubt must, however, remain about the subsequent loss of some of these nuclei, since our measurements of endothelial nuclear length are not really adequate in late degeneration, and the changes of populations are too small to be substantiated by our available material.

## SYNTHESIS OF NUCLEAR POPULATION CHANGES

In previous sections we have described the population changes of each cell-type, in each case taking the population of this particular cell in the undegenerated nerve as 100. The relative increases of the different cell-types on this basis are shown in Figs. 4 and 7 (taken from Tables 4–6). We have also given the percentage which each cell-type constitutes of all cells at different stages of degeneration, and this is shown in Figs. 5 and 6 (taken from Tables 4 and 5). Table 6 gives the composition by cell-type of a representative sample of 100 nuclei in the undegenerated nerve, and what this group of nuclei becomes as degeneration proceeds. Some of the stages from this table are illustrated in Fig. 8.

## DISCUSSION

We defer a general theoretical discussion of our findings until our quantitative data on Wallerian degeneration are more complete. In this section we consider a few points which call for comment.

### *Specificity of material*

We have limited this study to the tibial and peroneal branches of the sciatic nerve in the thigh of the rabbit. The changes of population during degeneration are extremely similar in these two branches. It is, however, unwise to attempt to generalize our conclusions to other nerves until we have made comparisons, since the literature suggests that the time relations of degeneration may vary considerably. We mention two points in this connexion: (*a*) the tibial branch is rather more than twice the size of the peroneal, which indicates that

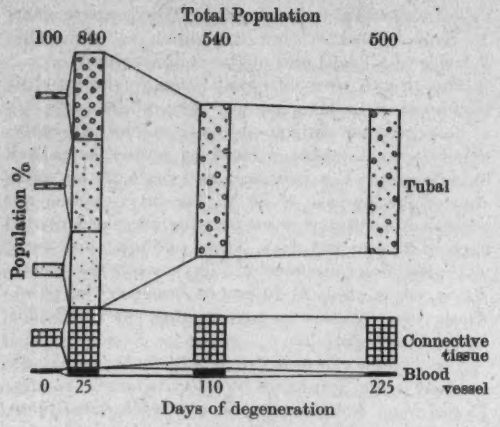


Fig. 8. Changes undergone by a representative sample of 100 nuclei of undegenerated nerve during subsequent degeneration. Large ○, medium •, and small · tube nuclei are shown separately at 0 and 25 days and subsequently combined.

hypothesis of chemical activation can obviously be extended to cover the vascular changes and macrophage mobilization in a degenerating nerve, and a close analogy with the processes of inflammation and repair of other tissues suggests itself.

We have given specific growth rates for the total population of nuclei, and they show a rather regular decline with time of degeneration (Table 3 and Fig. 3). Since we have suggested that autolysis of nerve fibres produces nuclear multiplication, it might be supposed that these growth rates give a measure of the concentration of autolytic substances. The nerve is, however, according to our analysis, highly heterogeneous with respect to growth and of changing heterogeneity. Further, the several distinct subpopulations of the nerve, especially those in different sizes of Schwann tube, clearly grow with a considerable degree of independence. In the circumstances we cannot infer from changes in the overall specific growth rates changes in the overall concentration of products of autolysis, or in activity of any controlling agent (see Gray, 1929). The specific growth rates of the whole population must therefore be regarded at present as purely descriptive. It is also safest to assume that the same limitation applies to the specific growth rates of the different cell-types, until the populations they refer to have been shown to be reasonably homogeneous.

Table 6. *The nuclear population changes which occur in a region of nerve containing* 100 *nuclei when undegenerated, analysed according to cell-type*

| | | Tubal | | | | Endoneurial | | |
|---|---|---|---|---|---|---|---|---|
| Days of degeneration | All nuclei | Total | Large tube | Medium tube | Small tube | Total | Fibroblast and macrophage | Blood-vessel nuclei |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
| 0 | 100 | 50 | 8 | 11 | 31 | 50 | 39 | 11 |
| 5 | 163 | 87 | 7 | 15 | 65 | 76 | 63 | 13 |
| 10 | 390 | 260 | 99 | 55 | 106 | 130 | 116 | 14 |
| 15 | 635 | 469 | 173 | 136 | 160 | 166 | 149 | 17 |
| 25 | 840 | 667 | 227 | 240 | 200 | 173 | 157 | 16 |
| 45 | 600 | 460 | — | — | — | 140 | 125 | 15 |
| 110 | 540 | 403 | — | — | — | 137 | 122 | 15 |
| 225 | 500 | 367 | — | — | — | 133 | 120 | 13 |

a considerable size range does not affect the nuclear population changes; (*b*) the nuclear population changes of any nerve will presumably depend at least in part on its 'fibre spectrum'.

*The nuclear population increase*

It is difficult to interpret the population increase in the endoneurium during Wallerian degeneration except as due to a chemical stimulus coming by diffusion from the autolysing nerve fibres. The interruption of the nerve fibres more than a centimetre away from the region we studied can hardly lead to this multiplication of nuclei by any other means. It is a natural assumption that the same stimulus produces multiplication in tubal nuclei. In a study in tissue culture of nerves undergoing Wallerian degeneration (Abercrombie & Johnson, 1942) we were likewise led to infer a diffusing activator to explain the similar outwandering activity of Schwann cells and fibrocytes. Such a

Our hypothesis of a diffusing activator requires that the tubal contents should influence endoneurial population growth. The general trend of connective tissue specific growth rates actually follows fairly closely that of tubal nuclei as a whole (Table 3 and Fig. 3), and is therefore apparently consistent with our hypothesis. The *close* correlation of endoneurial specific growth rates with those of tubal nuclei as a whole is, however, not a necessary consequence of our hypothesis, in view of the heterogeneity of tubal nuclei. For instance, maximal specific growth rate in the connective tissue might well be correlated with the intense multiplication in the large tubes; but our data

suggest that it coincides rather with that of the small tubes.

The present survey of nuclear population cannot hope to do more than raise these problems of the relations of the various cell-types. Their solution must await much more extensive data.

### *The nuclear population decrease*

At present we have not sufficient data to put forward a reasonable hypothesis as to the population decrease. We must, however, comment on its relation to reinnervation. It is not, unfortunately, possible to prevent entirely reinnervation in long-degenerated nerves, and it may be supposed that reinnervation leads to some loss of cells as the nerve returns towards normal. Actually there is no detectable correlation in our material, none of which is heavily reinnervated, between amount of reinnervation and size of population. Further, the major loss of population seems to follow closely the peak of population at 25 days; and this is not a period when reinnervation presents any problem. While therefore reinnervation may produce some of the slow loss of cells late in degeneration, it cannot explain the more important loss immediately after 25 days of degeneration.

### *Relation of population changes to outwandering activity of Schwann cells in tissue culture*

In our investigations of the outwandering activity in tissue culture of Schwann cells at different times of degeneration (Abercrombie & Johnson, 1942), we found that this activity rose from zero in the undegenerated nerve to a high peak at about 25 days of degeneration, then fell steeply until about 60 days and subsequently declined very slowly until up to a year. Clearly the outwandering activity closely follows the population cheangs of tubal cells (Table 4, column 3, and Fig. 4), and it is tempting to consider the outwandering curve as explained by population changes. There are, however, important differences, which suggest that the relation is more complicated, such as that the outwandering curve starts from zero, and shows a much more extreme rise and fall than the population curve.

## SUMMARY

1. Changes of nuclear population during Wallerian degeneration of rabbit peroneal and tibial nerves (excluding perineurium and epineurium) in the thigh region are recorded up to 225 days of degeneration. The population has been standardized for changes in nerve size and nuclear size and partly analysed according to cell-type.

2. The total nuclear population shows no change during the first 3 days of Wallerian degeneration; the growth curve then shows a sigmoid rise to a peak population about 8 times the initial population, reached at about 25 days of degeneration; and finally a fall, on a much smaller scale, so that at 225 days of degeneration the population is about 0·6 time that of the peak, and is equivalent to that of a nerve of about 12 days of degeneration (Table 2, Fig. 2).

3. The daily increment of nuclei (the additive growth rate) has its highest average value in the 10–15-day period of degeneration. It averages a little lower in the 3–10-day period, and considerably lower in the 15–25-day period. It reaches zero at about 25 days of degeneration. The negative additive growth rate of the population decrease has the highest average value near the beginning of the decrease (between 25 and 45 days), and falls thereafter. The trend of our figures suggests that it may not reach zero during the 225 days of degeneration that we have investigated (Table 3).

4. The growth of population relative to the number of cells present (specific growth rate) is highest at or near the beginning of the period of population increase, and declines to zero at about 25 days of degeneration. The negative specific growth rate of the period of population decrease has the same trend as the additive growth rate for that period (Table 3, Fig. 3).

5. The cells inside the Schwann tubes (Schwann cells, together with a relatively small number of macrophages at some stages of degeneration) account for most of these population changes. They increase in number about 13 times during the first 25 days of degeneration, and by 225 days have fallen again to about half of this peak value. They start at 50 % of all nuclei in the undegenerated nerve, rise to 79 % at 25 days, and fall to 73 % at 225 days of degeneration. The additive and specific growth rates of tubal nuclei show the same trends of change as those of all nuclei described above (Table 4, Figs. 3–5).

6. The tubal nuclear population has been analysed into three groups, according to diameter of the Schwann tube, over the period of population increase (Table 4, Figs. 6, 7). The average specific growth rate over the whole of this period is higher the larger the tube diameter. The additive growth rate is more nearly alike in the different sizes of tube, but is highest in the large tubes.

At the beginning of the population increase (3–5 days of degeneration) the nuclei of the small tubes (less than 4 $\mu$ diameter) have the highest specific growth rate of the tubal population. The nuclei of the large tubes (more than 9 $\mu$ diameter) are quite inactive at this time. The initial wave of multiplication in the small tubes is short-lived, and it is succeeded (starting at 6–7 days of degeneration) by a short wave of intense multiplication in the large tubes. The medium tubes (4–9 $\mu$ diameter) have a more diffuse period of high specific growth rate, with maximum probably situated in time be-

tween that of large and of small tubes. The nuclei of all three sizes of tube show a decline in specific growth rate after 10 days, reaching zero between 15 and 25 days. The maximum additive growth rate of small tube nuclei occurs at 3–5 days, of large tube nuclei at 5–10 days, and of medium tube nuclei at 10–15 days.

7. In the endoneurium fibrocytes and macrophages have not been distinguished but are treated as a single connective tissue group. They increase in number about 4 times during the first 25 days of degeneration, and by 225 days have fallen again to about 0·8 of this peak value. They start at 39 % of all nuclei in the undegenerated nerve, fall to 19 % at 25 days, and rise to 24 % at 225 days of degeneration. During the period of total population increase the endoneurial connective tissue nuclei increase in general like the tubal nuclei, with highest specific growth rate near the beginning of multiplication, declining to zero between 15 and 25 days. Unlike the tubal population, the highest additive growth rate of connective tissue nuclei is near the beginning of the period of population increase. The average specific growth rate for the period of increase is lower for connective tissue than for any of the three groups of tubal cells analysed (Tables 3, 5 and Figs. 3–5).

8. The number of blood vessels in the endoneurium shows no change during degeneration. Each $\mu$ length of blood vessels supplies in the undegenerated nerve about 0·4 cell; at 25 days of degeneration about 3·5 cells; and at 225 days about 2 cells.

9. The number of blood-vessel nuclei (endothelial and smooth muscle) increases a little during the period of general population increase, reaching about 1½ times its initial value by 15–25 days of degeneration; and perhaps decreases again during the period of population decrease. Blood-vessel nuclei start at 11 % of all nuclei in the undegenerated nerve, fall to 2 % at 25 days, and rise to 3 % at 225 days of degeneration (Table 5, Figs. 4, 5).

The expenses of this investigation were partly met by a grant from the Medical Research Council.

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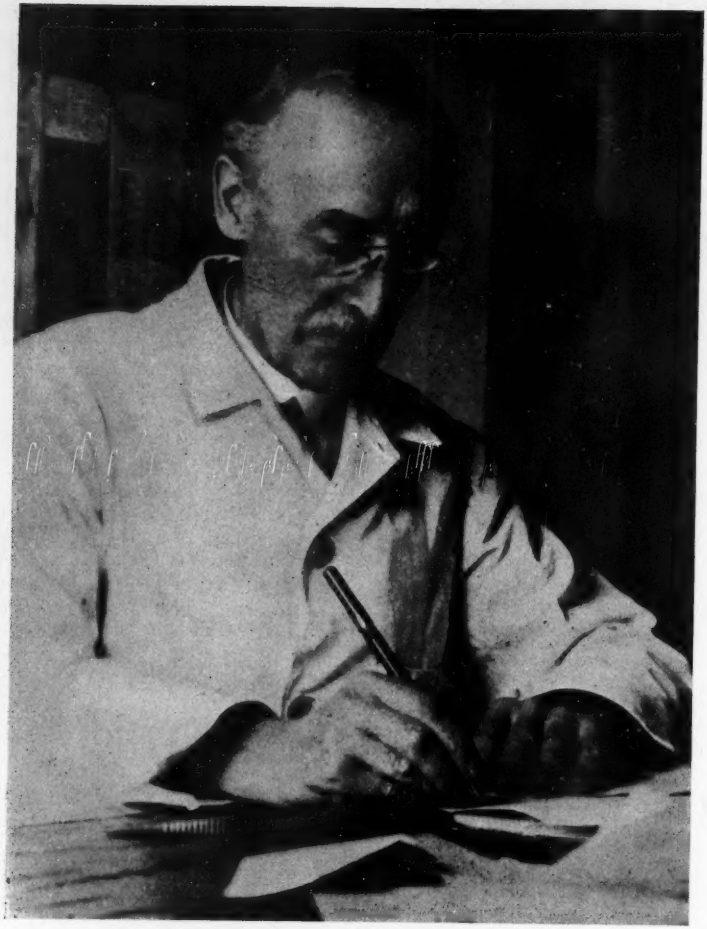

**EDWARD BARCLAY SMITH**

**1861–1945**

UNIV. OF MICH.

## IN MEMORIAM

### PROFESSOR E. BARCLAY SMITH, M.A., M.D.

1861–1945

Professor Barclay Smith, after a long illness courageously borne, died aged 84 on 5 July 1945, shortly after the death of his devoted wife whose loss was deeply felt by him during the last few months of his life. His home at Blackheath was destroyed by 'enemy action' at an early stage in the European war, and his latter days were spent in an hotel in London, to which he and Mrs Barclay Smith had removed in order to be near their two daughters who were engaged in war work in the neighbourhood. The hotel was in an area frequently exposed to the dangers of air attack, but he decided to remain there in spite of the attendant risks.

Barclay Smith will be long remembered by members of the Anatomical Society as their Honorary Secretary and genial friend, for he devoted a very large part of his time in promoting the well-being of the Society, and they will bear in mind his kindly welcome to visitors and to all new members. Apart from the social aspects of the Society, he served for many years on its Council and Editorial Committee, and he acted as Treasurer from 1931 to 1937. His genuine interest in the Society and more particularly in its younger members was evidenced by the kindly way in which he encouraged the attendance of those living at a distance by establishing—at his own expense and anonymously—a travelling fund to enable them to come to the central meetings. As another instance of his work for the Society, members will recollect the valuable work which Barclay Smith accomplished at the 'Reunion' meeting of the 'Association des Anatomistes' held in the University of London, when Prof. Brachet and other eminent foreign anatomists met members of our own Society in April 1927. The success of this and other meetings was largely due to the hospitality and devoted efforts of its Secretary, and also one would like to mention, of Mrs Barclay Smith, who so ably assisted and supported him.

Barclay Smith came from a military and naval stock, being the son of Captain W. E. Smith and Louisa Barclay, who was the daughter of a naval officer. He was educated at Monkton Combe School, near Bath, and at Brighton College. He commenced his medical training at Downing College, Cambridge, and completed his medical course at the London Hospital. Afterwards he became Demonstrator of Anatomy, and was subsequently appointed Lecturer in Advanced Anatomy at Cambridge under Prof. Alexander Macalister at a time when Sir George M. Humphry, the first President of the Anatomical Society of Great Britain and Ireland, still taught surgical anatomy in the old Anatomical Theatre there. In 1915, Barclay Smith was appointed Professor of Anatomy at King's College, University of London, succeeding Prof. D. Waterston, and he held this post until his retirement in 1927. During this period he served also as Dean of the Medical Faculty and Member of the Council, and was elected Fellow of King's College. He also held office in the University of London as Chairman of the Board of Intermediate Studies and the Board of Human Anatomy and Morphology, and was the representative of the Council of King's College on the Committee of Management of King's College Hospital. He examined in anatomy for the Universities of Cambridge, Durham, London, Manchester and Birmingham, and the Conjoint Board in England.

Prof. Barclay Smith's contributions to anatomical literature mostly appeared in the early stages of his career, between 1893 and 1911, and though mainly descriptive of osseous, muscular and visceral variations, included some interesting historical records and studies, such as that on 'John Halle: A Sixteenth-Century Anatomist.—The Compendious work of Anatomie, more utile and profitable than any heretofore in the Englyshe publyshed'—'perhaps the earliest anatomical treatise written by an Englishman and published in England now existent'. He also contributed a small booklet commemorating the first fifty years of the Anatomical Society. This is a valuable retrospect of the foundation of the Anatomical Society of Great Britain and Ireland in 1887 at the suggestion of the distinguished surgeon-anatomist Charles Barrett Lockwood, who, with the co-operation of Prof. Alexander Macalister and Sir George Murray Humphry, made the preliminary arrangements for its inauguration. It also includes an historical survey of the work accomplished by the Society up to 1937, with brief biographical notes of some of its more distinguished members during this period. For those who are old enough to remember the personality of the original members who were signatories of the 'attendance sheet' of the First Meeting of the Society, a facsimile reproduction of which is included in the brochure, these notes are intensely interesting, and bring back memories of many incidents which occurred not only at the demonstrations in the lecture theatres but at the informal luncheons and dinners, and at the excursions, which were arranged for the Summer Meetings of the Society. Our late Secretary took a very active part in organizing these meetings, and in promoting the

good fellowship which they engendered. No one could have put on record the various incidents alluded to in this brochure better than he, and he has done a signal service to the Society in its compilation. Of Barclay Smith's scientific contributions to anatomy, about seventeen in number, one of the most interesting and typical was that, published in the *Journal of Anatomy and Physiology*, 1911, entitled 'Multiple anomaly in a vertebral column'. It serves as a good example of the completeness of his work, his minute observation and descriptive ability. The anomalies described occurred in an Egyptian skeleton which was regarded by Elliot Smith as probably belonging to the Persian or Ptolemaic period, *circa* 600–500 B.C. In summing up the conclusions made from his observations, Barclay Smith stated that 'A careful analysis of the articular features in the cervical region shows that the reduced mobility which would of necessity result from vertebral fusions, was at least neutralized by the secondary articular modifications with which these fusions are associated. It may not be very wide of the mark to conclude that this spine evinces marked reaction to prolonged and excessive usage, that this excessive usage commenced at a fairly early stage of life, and that the possessor was a contortionist.' Appended to the concluding paragraph of the article is a note by Prof. A. Macalister, illustrated by outline drawings, showing the figures of two female acrobats, which he had seen in a tomb at Beni-Hassan. The figures represented show extreme forms of spinal contortion. Whatever view the reader may hold with regard to the suggestion that the skeleton belonged to a contortionist, the deduction made by Prof. Barclay Smith that compensation for loss of mobility, due to fusion of vertebrae in one part of the spinal column, may be obtained by the development of secondary changes in the form of adjoining articular surfaces, thus permitting greater mobility in another part is thoroughly sound, and an excellent illustration of the interdependence of structure and function.

Beside his scientific contributions to anatomy, Barclay Smith co-operated in editing Buchanan's *Manual of Anatomy*, and was a member of the Editorial Committee of the *Journal of Anatomy*. He edited the *Proceedings of the Anatomical Society* and rendered most valuable aid in supervising papers written by members of his staff in the Anatomy Department of King's College.

Barclay Smith was greatly interested in sport of all kinds. He was a life member of the Cambridge 'Rugger' and Cricket Clubs, and presented a cup for the King's College Rowing Club, when in London. At Cambridge, many years ago, he organized a very successful annual cricket match during the long vacation between the anatomists and physiologists, captained by himself for the anatomists and by Prof. Sims-Woodhead for the physiologists. He was a good golf player, and also a philatelist, and did a great deal to encourage young people in stamp collecting, which he considered a valuable aid to general education.

Barclay Smith will long be remembered by his friends of the Anatomical Society and at King's College, and the Memorial Service held for him on Saturday, 28 July, in the Crypt of St Paul's Cathedral, which was attended by a large number of his relatives and friends, and which was conducted by the Archdeacon of London and Dr W. R. Matthews, the Dean of St Paul's, was a fitting honour to one who had devoted himself so wholeheartedly to his work and official duties at King's College, and in connexion with the various Institutions and Societies to which he belonged.

R. J. G.

## GEORGE HENRY STANDISH LIGHTOLLER, M.D.

*Honorary Demonstrator in Anatomy, The University of Sydney*

1881–1944

On 4 May, George Henry Standish Lightoller died of heart disease. For 24 years he had carried out research work in the department of Anatomy of the University of Sydney. For part of this time he was in general practice and later a consulting physician.

An Australian descendant of an old Lancashire family, the outstanding facts of his life have been recorded elsewhere (*Medical Journal of Australia*, 5 Aug. 1944) and tribute paid to his many good qualities. I here wish to record briefly his scientific work, so much of which was published in this *Journal*.

Of his first four researches, three were done in collaboration with the writer upon the physical anthropology of the Australian aboriginal, more especially upon the facial muscles and soft parts of the nose. Suggestions were put forward as to possible correlations between the bony configuration of the floor of the nasal aperture and the teeth and the soft parts of the nose. Data were also given for a comparison of the facial musculature of the Australian aboriginal with that of other races, the main features observed being the coarseness and lack of differentiation, the marked development of a m. malaris and the extension of the labial tractor muscles to the lip edge. He next obtained his M.D. degree for a thesis, later published in this *Journal* (1925), upon the action of human facial muscles.

In this study, he employed slow-motion cinematography upon a patient with lipodystrophic absence of subcutaneous facial fat, so that the various facial muscles showed up most distinctly, especially in action. Among the many problems studied were those of the 'modiolus' region, a word which he coined, the action and structure of the mentalis muscle with a careful analysis of the depressor anguli oris, and the facial portions of the platysma; also the detailed structure and action of the orbicularis oris in various lip movements, and finally the action of facial musculature in sound production.

From this period (1926) Lightoller gradually extended his researches to almost the whole of the vertebrate field, as exemplified first in his paper on the 'Facial Muscles of the Orang Utan and two Cercopithecidae', and after a long period his monumental work emerged. This was entitled, 'Probable homologues. A study of the comparative anatomy of the mandibular and hyoid arches and their musculature' (*Trans. Zool. Soc. Lond.* 1939), later followed by various amplifications of this theme such as his final study 'Matrices of the facialis musculature. Homologization of the musculature in monotremes with that of marsupials and placentals', and three earlier papers, one on the homologization of the jaw musculature of sting-ray and two on the comparative morphology of the m. caninus (this *Journal*, 1940) and the platysma. His comparative study of the mandibular and hyoid arches comprised a detailed analysis of the constrictor muscles of the branchial arches in elasmobranchs (Euselachii), and then an attempted homologization with the branchial arch musculature in Amphibia, Reptilia and Mammalia. These comparisons were succinctly presented in a series of tables among which we may mention one showing the derivation of the m. stapedius and m. digastricus posterior from the matrix of the levator muscles of the 2nd branchial arch and his classification of the mammalian facial muscles. Also in one of the ancillary studies, on the musculature of the mandibular and hyoid arches in a sting-ray, he succeeded in establishing the complete and detailed homology of the musculature of the mandibular arch with that of a generalized posterior branchial arch system. In his main work he also gives a detailed discussion of the origin of the mammalian digastric muscle and made a contribution to our knowledge of the columella and ossicula auditus.

Most important of all perhaps is the experimental and other work which show conclusively that the superficial constrictor and levator muscles of the branchial arches in Euselachii are innervated solely by the Vth cranial nerve (1st arch), VIIth cranial nerve (2nd arch), and occipito-spinal nerves (remaining arches), while '*no branch from the Xth cranial nerve was found to supply the superficial constrictors and levators*'. This I consider one of his most important contributions to comparative morphology, backed as it was by masterly dissections and experiments on the living shark. It has profound significance when considered in conjunction with studies of Dr Addens of Amsterdam upon the motor nuclei and roots of the cranial and first spinal nerves of vertebrates and the occurrence of crossing root fibres in the facialis, vestibular glossopharyngeal and vagus nerves.

Such, in brief, is the record of scientific work of a general physician, who in 1920 decided to set aside one or more afternoons a week from a busy practice, and devote them to research.

A. N. B.

## Emeritus Professor J. T. WILSON, M.A., M.B., LL.D., F.R.S.

The death on 2 September of Emeritus Professor J. T. Wilson has taken from us one of the best loved and oldest members of the Anatomical Society. Professor Wilson had been a member of the Society since 1890, and had won a firm place in the affection and regard of all who knew him. His passing will be mourned by anatomists the world over.

The story of his life and work is a stimulus to the younger generation and a happy memory to his contemporaries. It is thus fortunate that we have it so fully and sympathetically described by his old friend Professor J. P. Hill, in Part I of the 'Wilson Volume' of the *Journal of Anatomy*, vol. 76, 1941, which was presented to him in celebration of his 80th birthday. We can in that account regain a picture of one whom we shall greatly miss.

## ANATOMICAL NOTE

### QUADRICIPITAL M. BICEPS BRACHII

By M. A. SHAH and M. SHAH, *Department of Anatomy, Dow Medical College, Hyderabad*

The following bilateral anomalous constitution of the m. biceps brachii was noted during the recent dissection in this Department of an adult male.

The origin of the muscle comprised: (1) the usual long or glenoid head; (2) the customary short or coracoid head; (3) a third or humeral head, lying deep to these and arising from the humeral shaft near the coraco-brachialis insertion; (4) a fourth or capsular head, arising from the front of the shoulder-joint capsule. Proximally this fourth head was a long slender tendon, deep and adherent to the tendon of m. pectoralis major: distally it was a fleshy belly, some three inches long, first between, and finally fused with, the bellies of the glenoid and coracoid heads. It probably represented a portion of the embryonic long (glenoid) head which had failed to become intracapsular. According to Le Double (1897) comparable examples of a quadricipital m. biceps brachii have been noted but rarely—once by Chudzinski and once by Kölliker.

In the same subject, a quadricipital m. pronator teres occurred bilaterally. In addition to the usual humeral and ulnar origins, the pronator received a third head from the medial intermuscular septum and a fourth head from a well-developed supracondylar process.

The brachial artery arose from the second part of the axillary: it ran superficially in the arm, crossing the median nerve and the biceps tendon, but pursued a normal course in the forearm. It was replaced in the arm, as regards both relations and branches, by the ulnar artery.

### REFERENCE

Le Double, A. F. (1897). *Traité des Variations du système musculaire de l'homme*, **2**, 45. Paris: Schleicher.